Therapy of Fungus Diseases

THERAPY OF FUNGUS DISEASES

An International Symposium

EDITED BY

THOMAS H STERNBERG, M D

Professor of Medicine (Dermatology) and Assistant Dean for Postgraduate Medical Education

AND

VICTOR D NEWCOMER, M D

Associate Professor of Medicine (Dermatology)

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The Division of Dermatology, Department of Medicine, School of Medicine and Medical Extension, University Extension, University of California at Los Angeles



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Foreword

The symposium reported in this volume was organized for the purpose of stimulating an exchange of ideas on both a national and an international level concerning the therapy of fungus infections It was held under the auspices of the Division of Dermatology Department of Medicine School of Medicine and Medical Extension University Extension University of California at Los Angeles

It is our feeling that the symposium was successful in achieving its primary purpose A total of 208 scientists participated representing 24 states and 8 foreign countries In addition to the papers presented an important aspect of the symposium was the establishment of personal contact among many of the participants who previously had known each other only by name It was necessary to limit the length of the papers in this publication because of the large number presented and therefore the majority are condensations of the material given at the symposium For similar reasons it was not feasible to include any of the discussions which contributed so much to the over all interest of the program In an effort to expedite publication reproduction of tables and charts was done by photographic process wherever feasible

This symposium was made possible by the financial assistance of the Squibb Institute for Medical Research To a large extent the success of the symposium was due to the care with which the Planning Committee (Drs Roger O Egeberg William L Hewitt David L McVickar Orda A Plunkett J Walter Wilson and Edwin T Wright) formulated the program The smoothness with which each session proceeded attested to the skill of the chairmen Drs Harvey Blank Norman F Conant Arthur C Curtis Chester W Emmons and Donald M Pillsbury The efficiency of the symposium operation as a whole was due to the planning and hard work of the Medical Extension Staff Gertrude H McSpedden

Susie Cartt Vivian Omerberg Patricia Martin and Macine Lustig We wish also to thank Ronald M Reisner for his contribution in the editing of this volume Finally we should like to express our appreciation to the staff of Little Brown and Company for making possible the early publication of the material

Thomas H Sternberg M D Chairman
Victor D Newcomer M D Co-chairman

Contents

Introductory Remarks by Donald M Pillsbury

It is perhaps improper for someone who is not primarily a mycologist to attempt to introduce this symposium on the therapy of fungus diseases Nevertheless any clinician who must be responsible for the treatment of patients suffering from superficial or deep fungus infections is all too keenly conscious of the general lack of specific and effective methods of dealing with such diseases Among all the microbial infections of man the diseases caused by fungi are perhaps the most difficult to modify in their course or to prevent The conquest of most bacterial infections is well known — though the tide of battle may turn against us if we continue to use antibacterial agencies with the profligacy with which they have been employed to date with the resultant emergence of resistant bacterial strains at a rate which is more rapid than that at which microbiologists and chemists can develop new antibiotics The Rickettsial and Chlamydozoaecae infections are one by one yielding either to chemotherapy or to prophylactic immunization Viral infections though not influenced by any specific methods of treatment can in some instances be completely prevented by immunization In the case of diseases caused by the superficial ringworm fungi on the other hand the methods of treatment are generally roundabout and non specific The modes of transmission of all except tinea capitis are relatively unknown and subject to much speculation and the available methods of prevention are poor In only two of the deep fungus infections blastomycosis and sporotrichosis are the methods of treatment reasonably satisfactory and even in these there is room for much improvement. There are no practical methods of immunization against any fungus infection

One of the best indices of the clinical importance of a particular group of diseases and of the dissatisfaction with available methods of controlling them may be found in the interest and concern shown by agencies which have a responsibility for the health of large groups of people I have participated in the deliberations of several of these agencies in respect to fungus infections and would like to cite some of this experience briefly

The experience of the armed forces with fungus infections during the war was most discouraging and the situation has improved but little since A major portion of the dermatologic disability during the war resulted from superficial ringworm infections or conditions which were misdiagnosed as such It was clearly apparent that the training of American physicians and laboratory workers in the diagnosis of superficial ringworm infections was inadequate Any inflammatory eruption which occurred on the feet or groin or any scaling dermatosis which produced ring shaped lesions was almost automatically diagnosed as a fungus infection At the beginning of the war there was great official confusion as to the preferable methods of prevention and treatment This was to some extent resolved after much discussion by the wide scale introduction of fatty acid preparations for prophylaxis and therapy Although these preparations are known to be very feebly antifungal *in vivo,* and rarely capable of curing a superficial ringworm infection they possess the great advantage of being almost completely non sensitizing and relatively non irritating The situation in respect to preventive measures was equally confused and a variety of powders and footbaths were variously employed Eventually it was agreed that prophylactic footbaths were useless if not actually harmful at times and that prevention was best achieved by foot hygiene which was as scrupulous as possible without resort to antifungal agents Since the war the problem of superficial ringworm infections has required continuing attention by the armed forces

Another disease from fungus infection namely coccidioidomycosis became a major disease problem during the war The units principally affected were armored forces which had trained in areas in which coccidioidomycosis was endemic This problem is well known to all physicians in California but the numerous instances of progressive coccidioidomycosis which were encountered

later represented a new and unusual experience to many medical officers There was absolutely nothing that could have been done about this either from the standpoint of prevention or treatment a situation which obtains to this day

In addition to the armed forces the Institute of Microbiology of the National Institutes of Health has long been keenly aware of fungus infections as a significant public health problem and of the great need for fundamental studies in this field However in vestigations of other more spectacular diseases have gained the support of the public and the Congress and it has not been pos sible to stimulate and support such studies as fully as is desirable Furthermore there are not many laboratory scientists who are competent mycologists and the number of proposals for studies along sound and original lines has been small to date Within the past few months this problem was considered at length by an *ad hoc* committee summoned at the instance of Dr Leonard Karel Chief Extramural Programs National Microbiological Institute It is hoped that eventually a larger number of fundamental studies in the field of mycology can be stimulated and that sup port for them will be forthcoming

The program of this symposium contains many titles which ex cite one s interest In the following brief remarks on what might be done to remedy the present situation I shall no doubt include something which has already been done and which will be re ported during the next two or three days As one surveys the pub lished reports dealing with laboratory or clinical studies of the mycoses the impression mounts that too many investigators are simply treading water in a very small and restricted pond Several examples of this may be cited There are myriads of reports deal ing with the botanical characteristics of the growth of fungi *in vitro* on various artificial media and they continue to appear Familiarity with this discipline is essential to anyone who pro poses to study mycologic diseases but as an end of and by itself it is unpromising There has been and is excessive preoccupation with the development of compounds for the topical treatment of superficial ringworm infections This cannot be expected to sub side completely because the phenomenon of a pathogenic organ ism growing on the very surface of the skin in a position where topical therapy might fully be expected to be effective offers a

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therapeutic challenge which can hardly be resisted It would also appear that there may have been excessive preoccupation with studies having to do with the recovery of fungi from diseased tissue particularly skin without sufficient emphasis upon determining whether or not the fungus is contributing to the disease process in question and if so to what extent Possibly the best examples of this are the numerous studies having to do with the recovery of Candida from the normal and abnormal skin mucous membranes and viscera with no certain means of determining whether the condition should be labeled moniliasis or if the Candida present should be regarded only as fortuitous saprophytes

It is my belief that the investigative approaches to the therapy of fungus diseases must be increasingly bold and imaginative This necessarily will involve basic studies which do not — indeed should not — have any prospect of immediate practical application In the following paragraphs I have attempted to list a few examples of approaches which appear worthwhile

What are the modes of transmission and the natural reservoirs of pathogenic fungi? The natural reservoirs of organisms capable of producing deep fungus infections offer a fascinating and important field of study In the case of a disease such as coccidioidomycosis the available knowledge of the natural reservoirs of the fungus has proved to have great practical usefulness though those working in the field are I am sure still dissatisfied with their knowledge of this problem All of the other deep fungus infections furnish similarly inviting fields of study As to reservoirs for the transmission of superficial ringworm fungi there is a lack of firm knowledge which is truly astonishing Something though not enough is known in regard to the contagiousness of tinea capitis Knowledge in respect to the ringworm fungi affecting the glabrous skin is extraordinarily nebulous It has long been assumed that the fungi which are associated with dermatophytosis of the feet are transmitted through showers bathroom floors swimming pools and so on Yet when these supposed sources of infection are painstakingly and scrupulously searched for pathogenic fungi they rarely yield any and then only a few organisms Familial transmissions of such infections are almost unknown in temperate zones Attempts to produce athlete s foot in man under what might be regarded as optimal conditions meet with either failure

or a short lived inflammatory reaction which is in no respect comparable to the natural disease All this constitutes a fascinating mystery on which little light has as yet been shed

Why are the superficial ringworm fungi necrophilic? As has been shown by many observers particularly in the series of studies by Kligman * the superficial ringworm fungi lack the capacity to invade living tissue When they invade the scalp and the shaft of the hair, they progress downward along the hair shaft only to the layer of parakeratotic cells and no farther On the smooth skin they can be demonstrated only in the stratum corneum never in the underlying living epidermal cells except in decadent and dying form in Majocchi s granuloma There is indubitably something in the living cell which completely stops the further growth of these organisms What is it?

What are the precise nutritional requirements for the growth of pathogenic fungi? In developing laboratory procedures to make a diagnosis of a fungus infection the efforts of most investigators have been directed toward providing a nutrient substrate which will furnish the best possible growth conditions for the fungus *in vitro* Much is known as to the varying characteristics of fungus growth with different types of media but the precise reasons for these are little understood This furnishes another inviting field of study but one which probably cannot be prosecuted adequately on the basis of ordinary botanical mycology but will require the techniques of enzymology and cytology The growth requirements of the pathogenic fungi have not received anything approaching the study which has been given to bacteria in recent years nor are the precise cytologic changes under varying conditions as well established

How do the superficial ringworm fungi produce their harmful effects? In many diseases of the skin and mucous membranes the recovery of a particular strain of fungus from the affected area still leaves the clinician in a quandary as to whether or not the fungus is causing the disease in question With many of the deep fungus infections there can be no question that the disease state is largely produced by the invader In some superficial infections (for example tinea capitis or chronic *Trychophyton rubrum*

* Kligman A M Tinea capitis due to *M audouini* and *M canis* A.M.A. Archives of Dermatology 71 313 1955

infections or vesicular and bullous eruptions associated with *T mentagrophytes*) there can be little doubt that the pathologic changes seen are due primarily to the fungus present though the precise disturbances in host parasite relationship may not be fully delineated Under other circumstances the role of the fungus becomes less certain This is seen in many chronic intertriginous eruptions of the feet from which a fungus may be isolated but in which other factors seem to be principally responsible for the inflammatory changes The situation in respect to Candida infections is even more uncertain This organism may be recovered from a wide variety of lesions of the skin and mucous membranes and from viscera as well but it is extraordinarily difficult to determine the degree to which it is contributing to the inflammatory changes seen if indeed it is contributing at all It is fashionable to regard many of the reactions to broad spectrum antibiotics as due to the addition of Candida to the microflora of the skin and mucous membranes but this may be regarded as highly uncertain as a rule

Why is topical therapy for the superficial mycoses so ineffective? As mentioned previously the general lack of specific chemotherapeutic antifungal compounds is nowhere better illustrated than in the diseases caused by the ringworm fungi There are I suspect few large pharmaceutical houses in the world where the synthesis and testing of agents designed for the topical treatment of the superficial mycoses is not being pursued One suspects that such studies are foredoomed to failure though this is a rash statement In any event they should be preceded by a better understanding of the penetration of fungicidal or fungistatic agents into the keratinous dead cells of the stratum corneum hair and nails Much more needs to be learned of the mechanisms by which fungi produce inflammatory changes Along with many others who have suffered disappointments in the clinical trials of such topical agents I suspect that the eventual curative agent will be administered systemically and will be something which will concentrate and persist in the epidermal cells However significant progress has been made in the development of antifungal antibiotics and one may indulge some hope that these like antibacterial agents will have curative effects when applied topically to superficial ringworm infections

What are the host parasite relationships? Fungus infections present an array of questions in relation to age and sex incidence and variability of course which constantly puzzle the clinician Why do some types of tinea capitis affect only children while others affect adults as well? Why are fungus infections of the feet seen with such rarity in children so uncommonly in adult females and so commonly in adult males? Why is coccidioidomycosis a benign self limited disease in some individuals and a progressive lethal one in others? Why does this infection become disseminated so much more commonly among Negroes than among whites? Why is histoplasmosis a common deep fungus infection in some regions productive of clinical signs in only a few of the individuals affected? Questions such as these might be listed almost indefinitely and admittedly they might be asked in respect to many bacterial and other infections concerning which we profess to know a great deal more than is the case with fungus infections

These random remarks have been made to give some indication of the extent and importance of fungus infections as a source of banal disabling or fatal disease in man and to cite a few examples of the fascinating array of investigative approaches which present themselves I am sure that all of us in this group which contains representatives from many lands are fully in accord with the need for a discussion of these matters and are most appreciative to the Medical School of the University of California at Los Angeles for arranging this symposium and to E R Squibb and Sons for supporting it

June 23 1955

Therapy of Fungus Diseases

SELMAN A WAKSMAN

Outstanding Problems in the Study of Antibacterial and Antifungal Antibiotics, with Special Reference to the Antibiotics of Actinomycetes

Among the major characteristic properties of antibiotics is their selective action against different micro-organisms The range of such activity has been designated as the antimicrobial or the antibiotic spectrum Some of the most important antibiotics that have found extensive application as chemotherapeutic agents are active against bacteria and actinomycetes but not against fungi On the other hand a number of antibiotics have now been isolated which are active against fungi but not against bacteria and actinomycetes There is also a group of antibiotics active against both bacteria and fungi

Four important substances — penicillin streptomycin the tetracyclines and bacitracin — which are produced by three different groups of micro-organisms namely fungi actinomycetes and bacteria are typical antibacterial agents Although they vary in origin and in chemical composition they have one thing in common a lack of activity against true fungi They are selective in their action against different bacteria Penicillin and bacitracin are active largely against cocci and gram positive rods streptomycin and neomycin are active against both gram positive and gram negative bacteria the tetracyclines are active against various bacteria rickettsiae and some of the larger viruses polymyxin is active against gram negative bacteria

The antibiotics which are highly active against fungi but inactive against bacteria and actinomycetes include cycloheximide fradicin rimocidin nystatin and candicidin These are also se

lective in their action against different fungi Some affect only yeastlike organisms others are largely active against filamentous fungi still others have a broader range of activity extending also to tumor cells

The antibiotics that are active against both bacteria and fungi include tyrothricin thiolutin streptothricin and clavacin

CERTAIN PERTINENT FACTS CONCERNING *ANTIBIOTICS*

(1) Antibiotics represent a great variety of chemical compounds which vary greatly in their composition and physical properties There is apparently no correlation between the chemical composition of antibiotics and their activity against either fungi or bacteria or both

(2) The selective action of both antibacterial and antifungal antibiotics against various micro organisms is to be looked for in the specificity of their mode of action on the one hand and in the composition as well as the specific metabolism of the sensitive cells on the other

(3) Selective activity of various antibiotics against different micro-organisms is not only qualitative but also quantitative Some antibiotics in very low concentrations as 0 001 µg/ml are able to inhibit growth of certain micro-organisms whereas considerably higher concentrations of other antibiotics such as 100 µg/ml are required to bring about growth inhibition of the same organisms

(4) One of the major limiting factors in the practical use of antibiotics as chemotherapeutic agents is their toxicity to animals The effect of an antibiotic on animal tissues varies greatly with the nature of the substance and frequently also with the nature of the animal thus pointing to the selective activity of the antibiotic against different cells of higher forms of life

(5) Differences in the action of antibiotics against bacteria as compared to fungi and against cells of lower forms of life as compared to cells of higher forms of life point to one major significant consideration namely that the mechanism of action of antibiotics against living cells differs greatly for the various antibiotics

(6) The development among sensitive organisms of resistance to

a given antibiotic points further to a change either in the nutrition of the cells or in the mode of action of the antibiotic against the particular cells

(7) Actinomycetes are more nearly comparable to bacteria than to fungi in the degree of their sensitivity to antibiotics which points to the close systematic and nutritional relationship of the actinomycetes to the bacteria

(8) Some organisms produce only antibacterial or antifungal antibiotics others produce both types of antibiotics *Streptomyces griseus* forms the antibacterial streptomycin and the antifungal cycloheximide *S fradiae* gives rise to neomycin and fradicin *S rimosus* to oxytetracycline and rimocidin

(9) Some of the antifungal antibiotics are formed readily in the broth and others are found only in the mycelium of the organism

(10) Certain antibiotics such as penicillin polymyxin actinomycin and neomycin may be produced either by the same organism or by different organisms in different chemical modifications

PROBLEMS AWAITING SOLUTION

Certain aspects of the formation of antibiotics such as the effect of the natural environment on the organisms producing antibiotics their role in the survival of micro-organisms under natural conditions and the mechanism of formation of antibiotics under artificially *controlled conditions must remain in the realm of* speculation Sufficient information has accumulated however as a result of recent extensive studies on antibiotics to justify certain broad generalizations which can be used as a basis for the following pertinent questions

(1) What is the mode of action of a given antibiotic against sensitive micro-organisms with special reference to its antimicrobial spectrum?

(2) What is the difference in the chemical and biochemical makeup of the cells of fungi as compared to those of bacteria and actinomycetes that is responsible for the difference in their respective sensitivities to various antibiotics?

(3) Is there any correlation between the chemical structure of an antibiotic and its mode of action against different micro-organisms?

(4) Is there a relation between the antimicrobial activity of an antibiotic and its toxicity to higher animals?

(5) Is there any correlation between the selective action of antibiotics against normal cells and against abnormal or neoplastic cells?

(6) If the high toxicity of antifungal antibiotics to animal cells is an indication of similar biochemical processes between such cells and fungus cells might it indicate that one of the promising fields of investigation for substances exerting a specific antitumor action is to be concerned with that group of antibiotics with specific antifungal activity?

These questions have a direct bearing upon our understanding of the activity of antibiotics Any answer would not only tend to throw light upon their potential utilization but would also help in the search for new compounds which still unknown at present may prove to be more effective than those already known

ISOLATION AND PROPERTIES OF ANTIBIOTICS PRODUCED BY ACTINOMYCETES

It has been said time and again that all the antibiotics that have so far found practical application as chemotherapeutic agents are a result of a hit-or miss program or a kind of trial and error set of procedures Whereas this interpretation applies to much of the work on antibiotics which has yielded some very striking results as in the discovery of penicillin one would hardly be willing to accept it when applied to the systematic screening programs in vogue in recent years The latter have been applied primarily to the study of the production of antibiotics by actinomycetes They have yielded highly satisfying results including a large number of effective and useful antimicrobial agents

Following the rediscovery of penicillin by Florey and Chain all the important antibiotics with the possible exception of bacitracin and polymyxin have been obtained from actinomycetes Virtually all of these were isolated in systematic screening programs One may begin with the isolation in 1940 of actinomycin a general antibacterial and antifungal substance and gradually proceed to the isolation of sarcomycin an antitumor substance in 1952 of tetracycline in 1953 and quite recently of amino-isoxazolidone

under the names of cycloserine and oxamycin During that period some two hundred preparations and pure chemical compounds were discovered Out of these at least a dozen compounds have already proved to be highly effective therapeutic agents One need only mention the various antibacterial agents notably streptomycin and its derivative dihydrostreptomycin chloramphenicol chlortetracycline neomycin oxytetracycline and tetracycline

TABLE 1

ORIGIN CHEMICAL NATURE, AND ACTIVITY OF ANTIFUNGAL ANTIBIOTICS

Antibiotic	Produced by	Chemical nature	Activ upon
		ACTINOMYCETES	
Actidione	*St eptomyc gris us*	Diketone	Y asts and fungi
Actinomycin	*Streptomyc tibioticus*	Chromopeptide	Bacteria and fungi
Antimycin A	*St ptomyc s* sp	Nitrog nous phenol	Yeasts and fungi
Fr dicin	*Streptomyc fradiæ*	Nitrog nous weak ba	Yeasts and fungi
Musarin	*St ptomy s* p	Organic acid	Fungi and bacteria
Streptothricin	*Strept myce l vendulae*	O ganic ba	Fungi and bacteria
Nystatin	*Streptomyce* sp	Polyene	Yeasts and fungi
Rimocidin	*St ptomyc rimo us*	Polyene	Y sts and fungi
Antimycoin	*St pt myces ur u*	Poly ne	Y s ts and fungi
Cand idin	*Str ptomyc g i us*	Polyene	Yea ts
		FUNGI	
Clavacin	*Aspergillus lavatu*	Unsaturated k tone	Bacte ia and fungi
Gliotoxin	*T ichod rma*	Contains sulfur and nitrogen	Bacteria and fungi
T ichothecin	*T ichothe ium o um*	Unsatu ated ketone	Fungi
Vi idin	*Trichoderma viride*	Contains ca bon hydrog n oxyg n	Fungi
		BACTERIA	
Eumy in	*B c llu ubtil*	Alcohol soluble	Bacteria and fungi
Pyocy nin	*P udomon* ae uginosa	*a* k tophena ine	Bacte ia and fungi
Hemipyocyanin	*P eudomonas a rugin sa*	*a* o yphenazine	Bacteria and fungi
Tyrothri in	*Bacillus br vi*	Polypeptide	Bacte ia and fungi

(4) Is there a relation between the antimicrobial activity of an antibiotic and its toxicity to higher animals?

(5) Is there any correlation between the selective action of antibiotics against normal cells and against abnormal or neoplastic cells?

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TABLE 3

ANTIFUNGAL ANTIBIOTICS PRODUCED BY BACTERIA

Act e p imarily against fungi		Act e against fungi and bacteria	
Substance	Organism	Substance	Org nism
Bacillomycin	B e btili	Fluvomycin	B subtilis
Eumycin	B e btilis	Polypeptin	B irculans
Fungi tatin	B ubtilis	Simpl xin	B simpl x
Myco ubtilin	B subt lis	To imycin	B ubtili

TABLE 4

ANTIFUNGAL ANTIBIOTICS PRODUCED BY ACTINOMYCETES

Prima ily n fungal ag n		Act ag nst bacte a and fungi	
Actinone	St ptomyc sp	Act nomycin	S. a tib oticus
Antimycin A	St pt myc s sp	Aur othricin	S. l l flavus
Antimycoin	S. aureus	Camphomycin	St eptomyc s p
As o in	S. an u	Endomycin	St ptomyc sp
Cacaomycetin	S. ca aoi	H lixin	Streptomyc sp
Candicidin	S. g i eu	M crocin	Mi mono po a sp.
Candidin	S. vi d flavus	Musarin	St eptom p
Ch omin	S. r bjori	Mycomycin	Nora d ldoph lus
Cycloheximide	S. g u	N g icin	Str ptomyc sp
Eumycetin	S. po peoch mog n	Thiolutin	S. albus
Fradi in	S. fradiae		
M ld n	S. phaeoch om g u		
Nystat n	S. nour		
Ol gomycin	S. di t tochromog n s		
Pha f in	S. phae f i n		
Rimocidin	S. rim u		
Rota ntin	S. r ticuli		
Trichomycin	S. hachijoen i		

* Al o a ti upon actinomy t s

erythromycin, carbomycin, and viomycin; the antifungal agents cycloheximide, thiolutin, trichomycin, nystatin, and candicidin; and the antineoplastic agents actinomycin, carzinophilin, azaserine, and sarcomycin. What other group of microbes, even if one includes all the bacteria, all the filamentous fungi, and all the higher or mushroom fungi, can compare with those?

DISTRIBUTION AND ACTIVITIES OF ANTIFUNGAL AGENTS

The existing information on the various antifungal agents was summarized in 1952 (Table 1). Of the ten preparations reported to have been obtained from actinomycetes, three were active against both bacteria and fungi, and seven were active against yeasts and fungi but not against bacteria. Of the four antifungal preparations produced by fungi, two were active against both bacteria and fungi and two against fungi only. The four bacterial preparations were active against both fungi and bacteria. Five of these antibiotics are examined in further detail in Table 2, where their characteristic spectra are given.

More recently, because of the growing interest in antifungal agents, a large number of compounds have been isolated from cultures of bacteria and actinomycetes (Tables 3 and 4). The bac-

TABLE 2

COMPARATIVE ANTIBIOTIC SPECTRA OF DIFFERENT ANTIFUNGAL ANTIBIOTICS

Amount of material, μg/ml, required to inhibit growth

Test organism	Cycloheximide	Antimycin	Fradicin	Nystatin	Antimycoin
Candida albicans	1,000	1	0.8	3.1	1.7
Cryptococcus neoformans	0.2		3.0	1.0	1.4
Trichophyton mentagrophytes	>1,000	>33	4.0	6.3	20.0
Trichophyton rubrum	1,000		1.6	6.3	20.0
Blastomyces dermatitidis					
yeast phase	>1,000			1.6	1.5
Histoplasma capsulatum			2.0	1.6	
Coccidioides immitis	>1,000		1.3	6.3	
Saccharomyces cerevisiae	10			3.1	3.0
Penicillium notatum		13	0.4	3.1	1.5
Aspergillus niger	20	33	2.4		
Nocardia asteroides	1,000				>100.0
Bacteria	0	0	0	0	0

terial products are largely polypeptides The substances produced by actinomycetes largely members of the genus Streptomyces belong to several types of compounds

CHEMISTRY OF ANTIBIOTICS PRODUCED BY ACTINOMYCETES

Our present knowledge of the chemistry of antibiotics produced by actinomycetes is summarized in Table 5 Among the antifungal substances some (cycloheximide candicidin) are active primarily against yeasts whereas others (fradicin oligomycin) are active largely against filamentous fungi There are also differences in the fungistatic and fungicidal properties of the various antibiotics Of the several compounds that possess both antibacterial and antifungal properties the streptothricin group is of particular interest since this is a basic water soluble compound and is not too toxic

On the basis of their ultraviolet absorption spectra the polyene antibiotics can be divided into two groups (1) comprising nystatin antimycoin chromin and rimocidin (2) comprising ascosin candicidin trichomycin and candidin The polyene nature of these and other antibiotics is brought out further in Table 6

Any correlation of the specific activity of antibiotics against dif

TABLE 6

CLASSIFICATION OF POLYENE ANTIBIOTICS

Antibiotic	Polyene moiety	λ of absorption maxima (mμ)		
Nystatin		292	304.5	318
Rimocidin		291	304	318
Antimycoin	Tetraenes	291	304.5	318
Chromin		292.5	305	320
Eurocidin		318	333	351
Fungichromin	Pentaenes			
Fungichromatin				
Flavacid		341	358	379
Mediocidin	Hexaenes	337.8	356	377
Candicidin		359.5	379.5	401.5
Candidin		363	383	406
Ascosin	Heptaenes	358	377	399
Trichomycin		364	384	406
Candimycin		362	382	406

TABLE 5

CHEMISTRY OF ANTIBIOTICS PRODUCED BY ACTINOMYCETES

A. Carbohydrate type molecule (amino sugar + acetal linkage)

1 Streptomycin group
2 Neomycin group
3 Erythromycin group
4 Rhodomycetin g oup (containing sugar + chromophore)
5 Puromycin group (containing sugar + purine or pyrimidine derivative)

B Polypeptide type molecule (amino acid + amide linkage)

1 Amino sugar also present (streptothricin)
2 Containing no sugars (cinnamycin)
3 Cont ining a chromophore (actinomycins)
4 Contain ng a pyridine derivative (pyridomycin)
5 Containing aromatic nonchromophoric moiety (levomycin)
6 Amino acid derivatives (azaserine cycloserine)

C Aliphatic acids

1 Containing polyenyne (mycomycin)
2 Containi g polyen (cand cid n)
3 H gh mol cular containing nitrog n (nig ricin)
4 Miscellaneous alicyclic or heterocyclic acid c compounds (actithiazic acid)

D Miscellaneous bases (anisomycin)

E Miscellaneous neutral compound

1 Chloramphenicol
2 Not chemically defined (griseoviridin)

F Pigments

1 Tetracycline g oup
2 Quinones containing sugar moiety (rhodomyc n)
3 Quinones containing amino ac ds (actinomycin)
4 Quinones with indicator prop ties (actinorhodin)
5 Quinones of the xanthomycin type
6 Quinones of the luteomycin type
7 Miscellaneous quinones
8 Thiolutin group
9 Miscellaneous pigments

G Miscellaneous compound of unknown structure

1 *Containing* C H O N
2 Containing C H O N S
3 Containing C H O Cl
4 Unknown composition

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Fu gichr min	Pentaenes			
Fungichrom tin				
Fla acid		341	358	379
M diocidin	Hexaenes	337 8	356	377
Ca dicidin		359 5	379 5	401 5
C ndid n		363	383	406
A c sin	Hept enes	358	377	399
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ferent micro-organisms or combination of organisms and their chemical structure is hampered at present by a lack of sufficient information concerning the exact chemical composition of the majority of these substances The structure of only two agents specifically active against fungi isolated from the actinomycetes is known to date out of the more than thirty compounds so far reported These are cycloheximide and thiolutin Still less information is available concerning the mode of action of these antibiotics against sensitive organisms as well as the reasons for the specific antibiotic spectra

PRACTICAL UTILIZATION OF ANTIFUNGAL ANTIBIOTICS

Beginning with tyrothricin penicillin and streptomycin which are primarily antibacterial antibiotics various other agents have found extensive application in the treatment of numerous infectious diseases in man and in animals It is sufficient to mention the tetracyclines which possess antirickettsial properties Trichomycin and nystatin two antifungal agents have recently been introduced into practical medicine streptothricin has also been used in a limited way but nothing has been heard of it recently

In the control of plant diseases certain antibiotics are beginning to find extensive application It is sufficient to mention streptomycin among the antibacterial agents The antifungal agents have so far found only limited practical use as is the case for cycloheximide Candicidin appears to have definite promise

Certainly the need for additional antifungal antibiotics is great and the possibility of finding such substances is highly promising

LIBERO AJELLO

Soil As the Natural Reservoir for Human Pathogenic Fungi

Impressive evidence is on hand in support of the thesis that many of the fungi capable of causing human disease exist as actively growing saprophytes in nature. From 1892 to the present twenty-one species of pathogenic or potentially pathogenic fungi have been isolated from non-living sources (Table 1). As a consequence few can doubt that soil is the ultimate source of the etiologic agents of many of the mycoses.

As demonstrated by Emmons, soil surveys are one of the most fruitful ways for discovering the natural habitats of the fungi that cause human disease. During the past five years 1113 soil specimens collected from widely scattered geographic areas have been examined in our laboratory for pathogenic fungi.

Two techniques were employed for this purpose. One method which has yielded *Histoplasma capsulatum* and other systemic

TABLE 1

PATHOGENIC OR POTENTIALLY PATHOGENIC FUNGI ISOLATED FROM NON-LIVING SOURCES

Absidia corymbifera	Hormodendrum pedrosoi
Absidia ramosa	Microsporum gypseum
Allescheria boydii	Nocardia asteroides
Aspergillus fumigatus	Phialophora jeanselmei
Candida albicans	Phialophora verrucosa
Candida guilliermondii	Rhizopus arrhizus
Candida krusei	Rhizopus oryzae
Coccidioides immitis	Sporotrichum schenckii
Cryptococcus neoformans	Trichophyton mentagrophytes
Epidermophyton floccosum	Trichophyton rubrum
Histoplasma capsulatum	

pathogens involved injecting the supernatant from soil suspensions intraperitoneally into mice with subsequent culturing of their liver and spleen Dermatophytes were sought by baiting plates of moistened soil with pieces of human hair From 1039 of the soils through use of the mouse procedure 72 isolates of *H capsulatum* were obtained as well as 19 strains of *Allescheria boydii*, 12 of *Cryptococcus neoformans* and 1 of *Candida albicans* By baiting 389 soil samples with filaments of hair 114 isolates of the dermatophyte *Microsporum gypseum* were recovered These findings along with the geographic location of the soil samples examined are presented in Table 2

TABLE 2
SOIL STUDY DATA

Locality	No of Samples	Pathogenic Species Isolated				
		A boydii	C albicans	C neoformans	H capsulatum	M gypseum
Tennessee *	710	15	1	5	67	30
Hawaii	100	1		1		23
Panama	100	1			1	36
Georgia †	79	2		2		13
Michigan ‡	44					5
Nigeria	26			1		
West Virginia ‡	16					2
Canada ‡	12					2
Maryland §	11			1		
Alabama	10			2		3
Venezuela §	5				4	
Totals	1,113	19	1	12	72	114
No of soils tested for each fungus		1 039	1,039	1 039	1,039	389
Per cent positive		1 8%	0 1%	1 2%	6 9%	34 1%

* Only 73 tested for *M gypseum*
† Only 44 tested for *M gypseum*
‡ Not tested for *H capsulatum*
§ Not tested for *M gypseum*

This group of surveys although limited in geographic coverage and numerical scope has given an insight into the global distribution of certain of the disease-causing fungi The United States is not unique in harboring such organisms in its soils Screening of

soils in most areas of the world undoubtedly will result in essentially similar findings One should not however expect to encounter the same array of species throughout the world for it is well known that some fungi have such critical ecologic requirements that their geographic distribution is necessarily limited

In addition some light has been cast on the ecologic requirements of human pathogenic fungi Studies carried out in Williamson County Tennessee with Zeidberg have revealed that areas frequented by chickens are especially favorable for the growth and proliferation of *H capsulatum* Approximately 39 per cent of soils gathered in chicken houses and chicken yards yielded this mold in contrast to a 13 per cent yield from soils gathered in other habitats (Table 3) In addition it was learned that protection of the soil

TABLE 3

INFLUENCE OF HABITAT ON OCCURRENCE OF *HISTOPLASMA CAPSULATUM* IN SOIL FROM WILLIAMSON COUNTY TENNESSEE

Habitat	*No of Samples*	*Recovery of Histoplasma capsulatum*	
		No of Positive Samples	*Per Cent Positive*
Chicken areas	54	21	38 9
Other areas	46	6	13 0
Totals	100	27	27 0

* Data derived from a group of so l specimens collected for chemical analysis

from the elements favorably influenced the occurrence of this fungus Chicken house soils were 46 2 per cent positive while chicken yard soils gave only a 20 per cent recovery (Table 4)

The influence of chickens on the presence of *H capsulatum* in soil is indirect since laboratory tests and field observations indicate that chickens are not carriers of this organism

The desire to determine what indirect influence if any chickens exert on the growth of this fungus has led to two types of investigation the chemical and physical analysis of positive and negative soils and a comparative study of the mycoflora of soils yielding *H capsulatum* and those negative for this mold

The physical and chemical studies not unexpectedly revealed that chicken area soils have a significantly higher organic and mois-

pathogens involved injecting the supernatant from soil suspensions intraperitoneally into mice with subsequent culturing of their liver and spleen Dermatophytes were sought by baiting plates of moistened soil with pieces of human hair From 1039 of the soils through use of the mouse procedure 72 isolates of *H capsulatum* were obtained as well as 19 strains of *Allescheria boydii*, 12 of *Cryptococcus neoformans* and 1 of *Candida albicans* By baiting 389 soil samples with filaments of hair 114 isolates of the dermatophyte *Microsporum gypseum* were recovered These findings along with the geographic location of the soil samples examined are presented in Table 2

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causes of mycetomas and rare cases of systemic disease is wide spread in nature In addition to the recoveries from Tennessee Hawaii Panama and Georgia (Table 2) this organism has been discovered in Maryland and Virginia soils by Emmons and in Canadian soils by Cain Cooke has obtained numerous isolates from a polluted stream in Ohio as well as from several sewage treatment plants

It is truly remarkable that only one dermatophyte *M gypseum* is isolated with regularity from soil The ease with which most dermatophytes grow in soil under laboratory conditions encourages one to believe that most dermatophyte species may exist as saprophytes in nature

The survey of soils for human pathogenic fungi has provided some information regarding the natural habitats of these organisms It is hoped that data obtained from such studies may ultimately lead to the development of rational control measures for the mycoses

ture holding capacity than soils from other habitats and that they are more acid It may be postulated that these and other properties of chicken contaminated soils may create an environment more favorable for the growth of *H capsulatum* than occurs in other types of soil

An analysis of the mycoflora of chicken area soils and those from other habitats has been initiated in an effort to determine what role competition among soil fungi may play in influencing the oc currence and development of *H capsulatum* in soil

TABLE 4

INFLUENCE OF SHELTER ON THE OCCURRENCE OF *HISTOPLASMA CAPSULATUM* IN SOIL

Source of Samples	*No of Samples*	Histoplasma capsulatum *Isolations*	
		No	*Per Cent*
Chicken house	39	18	46 2
Open chicken yards	15	3	20 0
Under house	26	5	19 2
In open	15	1	6 7
Under barn	3	0	—
Other	2	0	—
Totals	100	27	27 0

Isolation of *C neoformans* from soils collected in four of our states as well as Nigeria and Hawaii indicates that this unicellular fungus has a wide distribution and is a normal component of the soil s mycoflora Of the soils from which this organism was isolated 8 came from chicken areas 1 from a pigeon nest and 1 each from the edge of a pond a barn and a tractor shed Although these isolations are few in number they seem to point to a correlation be tween avian habitats and the presence of *C neoformans*

Di Menna s isolation of *C albicans* from soil was confirmed by the recovery of that fungus from soil collected in the basement of an abandoned house Since *C albicans* is thought to be a component of the normal flora of human beings and animals the question whether it exists passively in soil following introduction by animal carriers as Di Menna has speculated or actually grows in soil remains to be resolved

Convincing proof is on hand that *A boydii* one of the common

natural habitat The true ecology of these fungi can be established only by studying the environment where they occur in one area and not in another Preliminary studies of this nature have been made in only two organisms *Histoplasma capsulatum* and *Coccidioides immitis*

The two diseases histoplasmosis and coccidioidomycosis have much in common in that the primary pulmonary infections are initiated by the inhalation of air borne spores and frequently heal by calcification They differ widely in their distribution in the United States The two organisms responsible although both are soil borne require an entirely different environment

The endemic area for histoplasmosis in this country as evidenced by numerous cases of the disease a very high prevalence of histoplasmin reaction and soil and animal isolations of *H capsulatum*, is in the east central region The endemic center is in the lowlands near the junction of the Mississippi Missouri and Ohio rivers Here we find a warm temperate climate with high humidity and an average rainfall of 40 to 80 inches These soils are characteristically acid relatively shallow and of slow permeability with excellent water holding capacity

Zeidberg[1] postulated a soil theory based on the correlation of red yellow podsolic soils over areas which correspond quite closely with the regions in which the highest prevalence of histoplasmin sensitivity has been observed He does not attempt to determine what particular physical chemical or biologic characteristics make these soils or any soil a good or poor natural habitat for the fungus

Furcolow and Sitterley[2] express the opinion that the average rainfall may be the determining factor whereas Beadenkopf and Loosli[3] postulate that *H capsulatum* is disseminated in the Mississippi Valley by flood waters which carry the fungus in washed out soil which is later dispersed as wind blown dust Mochi and Edwards[4] state in their reviews of world distribution of histoplasmin sensitivity that the localization suggests an association of the fungus with large rivers running through relatively low country

It has been established that a high moisture content is certainly one essential for soils supporting the growth of *H capsulatum* This is shown by the fact that most soil isolations have been made from sheltered locations where the soil was not permitted to dry

O A PLUNKETT

Ecology and Spread of Pathogenic Fungi

A review of current literature on mycotic infections shows that most if not all of the fungi capable of producing mycoses in man *exist in nature as saprophytes in soil or on vegetation* and organic debris When these organisms are transferred to a parasitic habitat in man with a complete change of environment most of them change their mode of growth and reproduction A diphasic condition is thus brought about by a change of environment to one which is usually less favorable for their growth but which they can tolerate Many other saprophytic fungi of a similar nature which are not able to adapt themselves to a new environment by changing their growth habits fail to become pathogenic although their spores are prevalent in man s environment In the past in studying the ecology of disease emphasis has been placed on the relation of host to environment ignoring the influence of environment on the causal organism

The environment of the soil inhabiting fungi has many phases which may be classified as living and non living The chief factors of the non living environment are temperature humidity precipitation wind direction and velocity type of soil water holding capacity of soil and the chemical nature and organic content of soil The living environment consists of the microscopic and macroscopic fauna and flora in the soil the type of vegetation growing there and the animal life feeding on the vegetation These variable factors produce numerous ecologic conditions which in turn support various species each adaptable to its particular environment

In our laboratory studies it has been possible to determine under controlled conditions cultural requirements for maximum growth and sporulation of the pathogenic organisms but we must not assume that these organisms behave in the same manner in their

months of July August and September when dust is most prevalent Summer rains which tend to lay the dust reduce the number of infections by preventing the inhalation of air borne arthrospores It has been observed that a heavy rainfall during the winter results in a heavier production of arthrospores during the following summer and thus increases the incidence of infection

Smith et al [*] studying the incidence of coccidioidal infections at four army airfields in the San Joaquin Valley found a heavy incidence of infection in the southern part of the valley with a decrease as one proceeds northward In the southern area there is more arid uncultivated land than in the northern part this may play an important part in determining the distribution of *C immitis* in the soil Irrigation and cultivation change the environment by increasing the moisture content of the soil and the humidity

Soil isolations of *C immitis* have been made from many areas in southern California and Arizona most of them from virgin desert soil samples taken from the vicinity of rodent burrows Failure to isolate *C immitis* easily from cultivated soils leads us to believe that the fungus does not thrive so well in cultivated soils because of the increased organic content and the competition with other micro-organisms

Cattle sheep and dogs as well as certain species of rodents are infected with *C immitis* There is no evidence of transmission of the disease from one animal to another or to man Evidence from numerous epidemics of coccidioidomycosis definitely proves that infections occur through the inhalation of dust borne arthrospores

One recent epidemic is of considerable interest In May 1954 several anthropology students from the University of California at Los Angeles dug and sifted the soil from an old Indian camp site for relics and artifacts About two weeks later four of these students and one who cleaned the specimens after they were brought into the laboratory developed primary pulmonary coccidioidomycosis The site of the camp was an open faced cave hollowed out of a sandstone cliff ten miles south of Inyokern in a rough desert area with no habitations within ten miles

Soil samples taken from the site in June 1954 gave positive cultures of *C immitis* Soil samples taken from various other stations within a half mile radius of the cave were negative No rodents were taken at the cave site but 155 pocket mice and kangaroo rats

out Grayson and Furcolow[8] report measurements of temperature and humidity from two point sources from which infections occurred and cultures were secured Here the humidity reached above 95 per cent sometime during the day over an extended period while the temperature ranged from 49 to 80° F They state that general observation of other known exact point sources revealed evidence of frankly moist areas with the suggestion of high humidity

In a study of thirteen epidemics Grayson and Furcolow found a common place of exposure in which in the majority of cases large quantities of dust bearing infected spores were inhaled In four of these cases decaying wood was involved as a vehicle of infection A correlation between the number of lesions and the severity of illness on the one hand and intensity of dust exposure on the other was established both between epidemics and within a given epidemic

H capsulatum has been isolated predominantly from soils near human habitation such as barnyards chicken yards under dwelling houses etc In many of these soils chicken manure has been present Chickens are not infected by *H capsulatum* and cannot be considered a reservoir It is concluded that the chicken manure increases the organic content in the soil thus making it a better medium for the growth of the fungus

Many animals including dogs cats rats mice skunks raccoons woodchucks and ferrets are known to be infected with *H capsulatum* There is no evidence that man is ever infected from animals

The endemic areas for coccidioidomycosis are confined to the dry arid regions of the southwestern United States northern Mexico and certain areas of South America having a similar climate In these regions the soil is shallow sandy or gravelly with a low organic content and poor water holding capacity The seasonal rainfall varies from 2 to 15 inches with long periods of low humidity High temperatures and frequent high winds resulting in dust storms are encountered in most of the endemic areas

The incidence of infection is not evenly distributed over the whole endemic region but is localized in various small areas One of the largest of these areas is the San Joaquin Valley of California Here the highest incidence of infection occurs in the dry summer

trolled by treating the mine timbers Smith et al[8] found that by grassing paving roads and runways and oiling athletic fields the infection rate with *C immitis* was reduced by one half to two-thirds on army airfields in the San Joaquin Valley

Except for the dermatophytes which are transmissible from man to man or animals to man we have little knowledge of how fungus diseases are spread Cases of coccidioidomycosis and histoplasmosis occasionally occur in individuals who have never been near endemic areas Conidia cannot be air borne for long distances and remain viable Most animals infected with histoplasmosis do not travel long distances from their home areas It has been shown that *H capsulatum* can be isolated from vomitus urine and feces from infected dogs In this way new small endemic centers may be established in nearby areas if the ecologic conditions are favorable We have never been able to isolate *C immitis* from the feces of infected animals However it is known that the organisms will withstand passage through the alimentary tract If infected animals are eaten by predatory animals it is possible for the organisms to be transported to a new location It is also possible for cattle horses and sheep feeding on dust-covered fodder to harbor the organisms long enough for them to be transported for many miles where the spores may be deposited in fecal material It is also possible for infected individuals to infect soils in a new location by means of sputum Adequate proof of any of these suggestions is lacking and new endemic areas could only develop if the environmental conditions were suitable for the saprophytic growth of the organism

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4 Mochi A and Edwards P Q Geographical distribution of histo-

taken from an area half a mile distant were not infected. In October surface soil samples taken at the cave site were practically sterile and failed to yield *C immitis* It was thought possible that the intense heat of 120 to 130°F had destroyed the fungus near the surface One hundred and twenty five pocket mice and kangaroo rats taken at various points within a five mile radius of the cave were not infected

In April 1955 soil samples were again taken from the surface and at depths of 4 and 8 inches at the cave site Three of five surface samples were positive for *C immitis*, as well as two of four samples from 4 inches deep Samples taken at 8 inches from the surface were all negative The soil at this time was quite moist at the lower level Other soil samples from various points near the site were also negative for *C immitis* Ninety four rodents taken at this time were not infected

Further studies on this area are being conducted It would appear from the preliminary observation that this is a very small localized area in which the soil is infected and has not spread to adjacent areas as evidenced by the fact that no infected rodents or positive soil samples were obtained It also seems probable that although the arthrospores may be killed in the surface layers of the soil during the hot summer the mycelium survives at deeper levels but does not penetrate deeply into the soil

Sporotrichum schenckii and *Cryptococcus neoformans* have both been isolated from soil wood and vegetation Because of their worldwide distribution it appears that their environment is not so restricted as that of Histoplasma and Coccidioides No comprehensive ecologic studies have been made on these two organisms *Blastomyces dermatitidis* which has an endemic area limited to the upper central part of the United States has never been isolated from nature

Recent isolations of pathogenic species of dermatophytes have been made in our laboratory and elsewhere from lungs of rodents and from soil samples taken from beaches and rodent burrows

Ecologic studies of the saprophytic stage of pathogenic fungi correlated with skin testing will aid in identifying endemic areas and in determining the spread to new areas A knowledge of the natural environment may aid in the control of infections In the South African mines the epidemics of sporotrichosis were con

trolled by treating the mine timbers Smith et al[9] found that by grassing paving roads and runways and oiling athletic fields the infection rate with *C immitis* was reduced by one half to two-thirds on army airfields in the San Joaquin Valley

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J WALTER WILSON

Possible Approaches to the Therapy of Fungus Diseases

There is considerable evidence that the incidence of disease caused by pathogenic fungi is increasing In some instances this is absolute represented for example by the westward wave of epidemic *Microsporon audouini* tinea capitis in the United States which began about fifteen years ago and the more recent invasion of the *Trichophyton tonsurans* variety from the South and illustrated as well by the possibility that coccidioidomycosis may be spreading its endemic area by the contamination of the soil of new regions by wind or animal borne spores In other cases the increase in incidence is only relative since improved control of other diseases causes the percentage due to the activities of fungi to loom larger The treatment of fungus diseases is therefore likely to be of mounting importance in the future

Although the study of the mycoses the organisms which cause them and chemical methods directed toward their control has become ever more popular in recent decades it has as yet afforded but little advancement in our methods of treatment of such infections The simplest conceivable way in which complete control of all fungus disease could be achieved would be through the discovery of a perfect fungicide Such a substance should have many attributes to be ideal it should be stable soluble colorless odorless tasteless absolutely nontoxic to human beings free of undesirable side effects and active against all pathogenic fungi wherever they might be in concentrations easily attained and maintained by oral or parenteral administration To reach such a goal would indeed require a constellation of fortuitous circumstances far beyond the anticipation of any serious scientist and yet an astounding amount of effort has been expended in this direction

Of thousands of medicaments intended to suppress the tineas and all too frequently erroneously claimed to be able to do so none can be truthfully said to have earned a place beside the wonder drugs such as those now available in other fields of medicine neither has any of the scores of compounds tested as yet achieved such status in the treatment of the deep mycoses In both these classifications it has been especially discouraging to observe that the more closely a chemical has approached reliable fungicidal efficiency the greater has been the incidence and severity of toxic reactions in the human being on or in whom it has been used This trend long ago became evident also in the antibiotic field since the same screening of the soils of the world which yielded so many marvelous and reasonably safe antibacterial weapons furnished only some antifungal compounds fairly effective in vitro but tending to be too toxic for parenteral use in human beings

Such compounds have also had other disadvantageous features They have usually been almost insoluble highly unstable unless kept in highly purified powdered form away from light and heat and highly colored It is probable that the somewhat higher position among the natural forms of life occupied by the fungi makes them less susceptible to drugs than their simpler relatives and more likely to be harmed only by medicaments tending to be toxic also to higher animals and man

For a time it seemed logical to expect the discovery of chemical compounds which could effectively cure the tineas by topical application in spite of the fact that they might be too hazardous for internal administration Since these superficial mycoses represent all but a small percentage of human fungus infections such medicines would solve the greatest portion of the problem leaving only the occasional case of a severe or fatal deep mycosis However it has become painfully evident that no drug yet devised can penetrate reliably the keratin of skin hair and nails so deeply as can pathogenic fungi At best such medications can do no more than slow down the rate of penetration of fungal elements into keratin and its predecessors enough to allow it to be exceeded by the rate at which such structures grow toward the surface thereby pushing the fungus entirely away from the body to be eventually shed

Failure to realize that this mechanical barrier can defeat the attainment of success by any chemical however potent as a fungi

cide has led to the use of stronger drugs in ever stronger concentrations until instances of local irritation have been frequently encountered and in a few instances serious toxicity within the body has occurred by absorption from topical administration I have emphasized my preference for long continued persistence in the employment of a changing series of reasonably antifungal preparations rather than reliance on an intensified use of any one such material since in my opinion none has emerged as superlative

Since topical medication has such limitations the ideal antifungal drug even for the superficial mycoses would seem to be one which could be safely administered internally in amounts sufficient to endow the cells eventually destined to produce keratin with power to resist fungi completely this power persisting as they become keratinized and the drug thus exerting its effects from within outward Although it would be unwise to advocate abandoning the search for such a panacea it seems unlikely that such a goal can soon be reached for once again as with the deep mycoses the probability of associated toxicity appears large

The financial success which would result from the discovery of a truly effective safe universal fungicide has provided a lure sufficient to cause too large a majority of the support available for research in mycology to be directed along this line For the same reason I am sure that nothing which might be said or done here is likely to cause this phase of the program to be harmfully neglected in the future It seems wise therefore to emphasize at this time that the eventual successful control of fungus infections may not lie in this direction at all and to plead for the expenditure of a greater proportion of research effort in investigating other approaches to the problem

The suppression of fungal activity within the body by means of a chemical does not necessarily imply that it must be directly toxic or poisonous to the microbe it may only need to enter into its metabolism and cause some change incompatible with its continued virulence such as that which explained the fungistatic ability of the topically applied fatty acids or the mechanism of the action of the sulfonamides It is entirely possible that some substance not actually a drug perhaps can be found which inhibits the growth and reproduction of fungi without any associated toxicity Several studies have recently revealed certain susceptibilities on the part

of certain organisms to deficiencies in one or more vitamins From such studies a specific treatment for a specific infection might be developed

One investigation even suggested that increasing the glucose concentration of the skin could combat otherwise intractable *Trichophyton rubrum* infection Although this was advocated as a topical procedure only it is interesting to consider its employment by systemically induced change in glucose content. The possibilities of approaches such as these should be more thoroughly studied even though at present it seems doubtful whether any such change effective against fungi could be induced in the human body in a degree sufficient to be curative without some danger

In the control of the tineas it is still possible although apparently remotely so that an antifungal element could be driven sufficiently deeply to be effective by the assistance of galvanic iontophoresis So far this modality has always proved more adequate in theory than in practice but it should not be entirely forgotten Since in many instances keratin provides a barrier to the penetration of fungicides to an appropriate depth improvements in keratolytics might help It is even possible that chemical or electronic measures capable of causing shedding of keratin structures such as hairs when applied locally might be developed to a successful degree One chemical was observed by me and my colleagues to cause epilation of the scalp resulting in cures in many cases of resistant *T tonsurans* infection Unfortunately it became apparent that some of this activity was due to systemic absorption and alarming although so far not serious neurotoxicity was encountered Consider for a moment how advantageous it would be if a method could be developed by which those nails involved in a fungus infection could be nontraumatically caused to be shed temporarily and completely without sequelae

Perhaps this same result could be obtained by another approach Since the rate of growth of keratin structures toward the surface of the body can result in the cure of a dermatomycosis if it exceeds the rate of penetration of the fungi inward measures to increase the rate of hair or nail production would be distinctly beneficial There has as yet been only one suggestion along this line the administration of thyroid extract It would be interesting to explore

the subject of hair and nail growth and the factors controlling the rates of each

However the most important of all such theoretical approaches to treatment stems from the realization which has become ever more certain in recent years that a serious or intractable fungus infection occurs only when there is something abnormal about the patient rather than because of an enhanced virulence on the part of the microbe The statistically significant association of the incidence of certain of the deep mycoses such as cryptococcosis and histoplasmosis with abnormalities in the reticuloendothelial system cannot be ignored Even though no such relationship has as yet been discovered in coccidioidomycosis it is amply evident that this disease reaches serious proportions only in those few individuals who are immunologically defective in some manner probably even before the infection was acquired

As another example some similar mechanism must underlie the fact that many individuals have been observed to be infected extensively by *T rubrum* continuously for many years without infecting the spouse or others in the family except occasionally a child who might thus be assumed to have inherited the significant defect Also moniliasis presents many aspects indicating lowered individual resistance

Thus the study of immunology may reveal methods by which these abnormally susceptible individuals can be returned to good health not by killing their fungal enemies but by rendering them normal in their ability to resist the infection This is an extremely complicated subject and one in which I myself have expended a great deal of effort without as yet having seen the least glimmer of light but I am all the more convinced that some highly intelligent scientist will some day make a tremendous contribution along this line

Aside from studies directed toward discovering how the patient deviates from the normal in the possession of natural immunity toward fungus diseases or toward the development of immunity by natural means the possibility of accomplishing the same result by artificial methods cannot be entirely ignored There is certainly no more reason to be discouraged by the lack of success attained in the past with vaccines antiserums and hormones than with that

gained from investigations of antifungal chemicals Perhaps there is still hope that an active immunity (or at least a passive one) can be artificially induced

In the past the greatest handicap which such nonfungicidal studies as these have had to face has been the difficulty of obtaining financial support they have usually been looked upon as too esoteric too impractical too lacking in commercial possibilities to warrant being underwritten by the pharmaceutical industry Even governmental and philanthropic institutions have become interested but rarely and with difficulty The application of the words basic research to such a project has usually sounded its death knell

It is difficult however to escape the conclusion that regardless of the type of approach which eventually proves to be ideal in the treatment of fungus diseases it must of necessity entail the employment of some sort of materials be they strictly chemical or biologic in nature There will therefore be opportunity for some degree at least of commercial exploitation simply because the services of commercial outlets cannot be dispensed with

Thus it would appear advisable to distribute the research effort in mycology more equitably in all of the directions where possible success might be attained rather than to channel it as in the past so predominantly in the direction of chemical fungicides lest the achievement of the ultimate goal in such an important field be unnecessarily delayed

DAVID L McVICKAR

An Evaluation of the Laboratory Methods for Testing Fungicides

None of the antibiotics employed so effectively in the treatment of bacterial and rickettsial infections are effective in the treatment of mycotic infections systemic or superficial

In general the attributes required of a fungicidal or fungistatic agent do not differ greatly from those required of an antibacterial agent Such compounds should be (1) water soluble preferably (2) capable of being transported to and penetrating into infected areas (3) capable of being maintained at an effective therapeutic level in the infected tissue — and this does not necessarily mean that the plasma content must be at this level and (4) nontoxic even on long-continued administration No antifungal substance with these characteristics is yet available

For the most part the in vitro techniques which have been developed for testing the antifungal activity of compounds parallel closely those developed for testing antibacterial agents with suitable modifications for the comparatively slow growth rate and more highly developed morphology of fungi Thus we have tests in which pathogenic fungi are grown in the continuous presence of a compound either in nutrient broth or nutrient agar in agar cultures the organisms may be either distributed evenly throughout the medium or restricted to a thin layer on the surface of the medium The compound to be tested may be incorporated directly into the medium whether liquid or solid however when an agar medium is used the compound can be confined to a small area through the use of impregnated disks or strips of absorbent paper Much useful information has also been gained through testing the antifungal activity of substances by manometric techniques and in experimentally infected animals

Since we are still in a sense in the pre penicillin era of the treatment of mycotic infections it is perhaps natural that the necessity for standardization of in vitro tests although well understood as a result of studies on antibacterial agents has yet to receive the attention it merits in the testing of antifungal compounds In the screening of large numbers of compounds for antifungal activity the need for meticulously standardized procedures is possibly not of paramount importance

Certain minimal requirements should however be met (1) The medium must support optimal growth of all organisms (2) It should contain adequate amounts of serum or albumin (3) Several different fungus pathogens should be used in particular neither *Candida albicans* nor *Cryptococcus neoformans* should be used as the sole test organism since both these fungi appear to be somewhat less sensitive to antifungal compounds than the pathogens which possess a distinctly mycelial phase of growth (4) In so far as possible the compounds should be tested on organisms which are cultured in their in vivo phase of growth it seems clear that the metabolism of the in vivo yeast cell phase of *Blastomyces dermatitidis* for example must differ greatly from that of the mycelial phase of this fungus

Parenthetically it might be noted that compounds should not be rejected because their antifungal activity is of a low order that is cannot be expressed in terms of a few micrograms per milliliter It is not unlikely that had the sulfonamides been investigated after rather than before the discovery of penicillin they would have been rejected Furthermore until a really potent antifungal agent has been found it may well be that excellent therapeutic results could be achieved by combining two or more substances of comparatively low antifungal activity Apparently such a procedure has not yet been tried

When it becomes necessary to investigate exhaustively the activity of one particular compound or to make a detailed comparison of the activity of two or more such agents complete standardization of all procedures is mandatory In addition to the requirements listed above the following conditions must also be met (1) The medium must support the growth of isolated single cells this is of particular importance when *Histoplasma capsulatum* is used as a test organism (2) *More than one strain of any given pathogen*

must be tested (3) The organisms must be standardized individually with respect to age of culture from which obtained and especially with respect to the concentration used (4) The test should be a quantitative one and should include the element of time

It must be admitted in conclusion that there is at present no proof of the need for complying with certain of the standardization procedures discussed well controlled experiments to establish the point have not yet been carried out The lack of information in this field reflects a general dearth of knowledge concerning the fundamental metabolic processes of the pathogenic fungi

A N CHAKRABORTY

Studies on Mycotic Diseases in India*

The climate of India varies widely because of extreme variations in physiography of the subcontinent High mountains forests vast alluvial plains and semi desert areas a long sea coast — all provide a variety of environmental conditions

Mycotic diseases vary in type and incidence in various parts of India and in different seasons In hot and humid areas different types of ringworm infection are common The superficial mycoses constitute the vast majority of diseases caused by fungi and most of these are correlated with climatic factors occupation and habits Fortunately the deep mycoses appear to be uncommon but their true incidence can only be determined by further study

THE SUPERFICIAL MYCOSES

An analysis of 12 002 patients with superficial mycoses attending the skin clinic of the Calcutta School of Tropical Medicine during the last five years (1950–1954) was made with respect to sex age and meteorologic conditions The results were as follows

SEX AND AGE INCIDENCE

The number of male cases was about double that of the female cases In the age group 0–4 years the number of male and female cases was about the same while in the age group 5–14 years the female cases were more numerous In the age group 15 years and over males predominated These features were present during the entire five year period

Since the cases were largely drawn from the population of the city of Calcutta the effect of sex and age was also studied by relat

I am indebted to Dr C. Chandrasekaran of the All India Institute of Hygiene and Public Health Calcutta for analysis of some epidemiologic data and a report including Tables I 2 and 3

TABLE 1

SEX AND AGE DISTRIBUTION OF 12 002 OUT-PATIENT CASES

Age Group (yrs)	Number			Per Cent		
	Male	Female	Total	Male	Female	Total
0–4	135	136	271	1 1	1 1	2 2
5–14	444	653	1 097	3 7	5 5	9 2
15–29	4 275	1 766	6 041	35 6	14 7	50 3
30 and over	2 852	1 741	4 593	23 8	14 5	38 3
Totals	7 706	4 296	12,002	64 2	35 8	100 0

ing the number of cases to the 1951 census population (The limitations of using hospital data in studying incidence are well known and also apply to this data) The number of cases per year per 10 000 population of specified sex and age group given in Table 2 show a somewhat different picture from that stated above The incidence among males and females was about the same in the age groups 0–4 years (2 8) and 15 years and above (13 7) In the age group 5–14 years however the female incidence was higher (7 7 for females and 4 6 for males) The incidence was found to increase with age

RELATIONSHIP TO WEATHER CONDITIONS

In Calcutta minimum and maximum temperatures minimum relative humidity and rainfall vary considerably from month to

TABLE 2

NUMBER OF CASES PER 10 000
POPULATION OF SPECIFIED SEX AND
AGE GROUP (ANNUAL BASIS)

Age G oup (yrs)	*M le*	*Female*	*B th Sexes*
0–4	2 8	2 7	2 8
5–14	4 6	7 7	6 0
15 and over	13 2	15 0	13 7
All ages	11 2	11 6	11 4

The figures fo both sexes and all ages have been obtained by the following calculation

$$\frac{\text{Number of average cases per year of both sexes and specified age group}}{\text{Population of both sexes and spec fied age group}} \times 10\,000$$

month while maximum relative humidity shows little variation The number of cases per month was correlated with the meteorologic data for the same month All the correlations were positive showing a rise in the incidence with an increase in the intensity of the meteorologic factor and vice versa (Fig 1 and Table 3) The highest correlation of 0 67 was seen with minimum humidity Correlation coefficients were also worked out allowing one month for the effect of the meteorologic condition to be reflected in the inci

TABLE 3

CORRELATION COEFFICIENT * OF MONTHLY NUMBER OF CASES WITH METEOROLOGIC FACTOR OF THE CURRENT AND PREVIOUS MONTH

Meteorologic Factor	*Current Month*	*Previous Month*
Maximum temperature	0 01	0 21
Minimum temperature	0 43	0 45
Maximum humidity	0 22	0 03
Minimum humidity	0 67	0 39
Rainfall	0 45	0 63

The correlation coefficient can take values from − 1 to + 1 Values from − 1 to 0 show negative correlation and 0 to + 1 show positive correlation The higher the value of the correlation coefficient in the range of 0 to + 1 the greater the degree of positive association

dence of the disease that is the meteorologic data for January were correlated with the number of cases in February and so on Rainfall gave the highest correlation coefficient 0 63

INCIDENCE OF DIFFERENT SPECIES OF FUNGI

The relative incidence of different species of fungi responsible for the superficial mycoses of glabrous skin in India and other countries is shown in Table 4[1] It can be seen that certain species are responsible for the majority of cases in each country

(a) *Ringworm of the scalp* is rare among Indian children It was found among English and Jewish children residents of boarding schools in hill stations and the causative organism was *Microsporon audouini*[2-4] These children were not exposed to infection outside India and had not been in contact with children recently arrived from abroad The remaining cases of ringworm of the

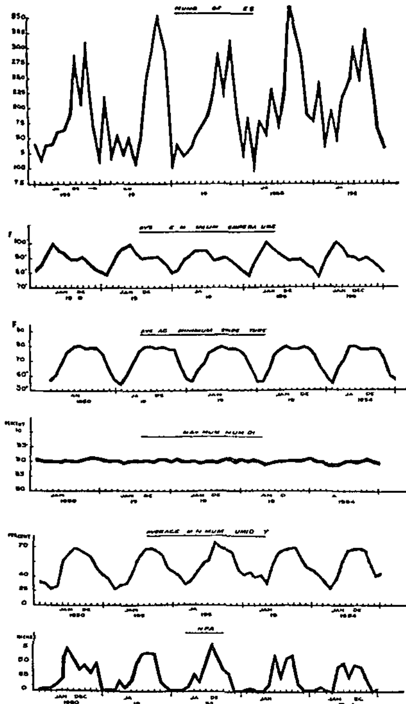


FIGURE 1
Graph of the Monthly Incidence of the Disease and the Meteorologic Factors – Calcutta 1950–1954

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ing this condition is difficult to culture A K Banerjee of my de partment has recently succeeded in growing *Microsporon furfur* on the surface of modified Petroff s medium It develops as a dry chalky or pinkish beadlike colony Subcultures are difficult to maintain

(e) *Animal mycoses communicable to man* In the course of recent investigation on this subject we isolated fungi from 13 of 137 animals examined *T rubrum* infection was observed in 4 of these 13 animals (1 dog 1 cat 2 cattle) The infection was con fined to the skin and the hair was unaffected The fungus grew readily in culture and these strains grew more rapidly than those isolated from man By applying the culture to lightly scarified hu man skin a ringworm infection could be produced [15 16]

From one of the 13 animals (1 cow) a species of Actinomyces that was identified as *A bostroemi* Bald was isolated [16]

THE DEEP MYCOSES

As already indicated cases of the deep mycoses are rather un common in India However sporotrichosis rhinosporidiosis and nocardiosis are occasionally seen in our clinic

Incidentally a strain of Sporotrichum *S schenkii* was isolated from a lesion in a cow The fungus was found to be pathogenic to both guinea pigs and rabbits It was also shown to be infectious to man after subcutaneous injection

Recently a case of histoplasmosis in man was reported from this school [17] A survey [18 19] using histoplasmin in patients with respira tory complaints revealed three positive reactions out of a total of 61 cases tested but the probability of a nonspecific reaction was sug gested by the coexistence of moniliasis tuberculosis or dermato mycoses in these cases A case of North American blastomycosis in volving the skin the right clavicle and the liver has also been re ported from Bengal but such cases of histoplasmosis and blastomy cosis are distinctly rare [20]

TREATMENT OF SUPERFICIAL MYCOSES

In India prevention and cure of the superficial mycoses is com plicated by the following factors The lesions occurring in the in digent population are often extensive and inflammatory Some are

scalp described from India were caused by *Trichophyton violaceum*,[3,5] *T gypseum*,[6] *T crateriforme*,[7] and *M ferrugineum*[2] *T gypseum* was observed among Gurkha soldiers during World War II[2,8]

(b) *Favus* is found among the people of the northwestern regions of India *viz* the mountainous areas of Kashmir the plains of Punjab and the semi desert areas of Rajasthan Occasionally a

TABLE 4

COMPARATIVE INCIDENCE OF SPECIES OF RINGWORM FUNGI INFECTING THE GLABROUS SKIN IN DIFFERENT COUNTRIES

Country	*No of cases*	*T rubrum (per cent)*	*F floccosum (per cent)*	*T gypseum (per cent)*	*T violaceum (per cent)*	*Other species (per cent)*
India	700	63 00	33 23	2 36	1 41	—
China	—	81 58	7 90	—	10 52	—
United Kingdom	—	29 00	44 50	26 50	—	—
United States	—	18 33	3 25	75 42	—	3 00

few imported cases are seen in Calcutta A new species of fungus *Achorion actoni*, was isolated from such cases of favus[9,10]

(c) *Actinomycotic lesions of the hands and feet* Castellani[11] described a peculiar pitting of the hands and feet and considered it as a manifestation of yaws Later it was found that it was due to a fungus *Actinomyces keratolytica* It is commonly seen among the indigent population of Bengal What actually happens is that the horny layer is dissolved or eroded by the fungus which produces pits or fissures that coalesce to form deep pits and furrows hence the species name In Bengal the condition is generally known as *chaluni*, meaning sieve owing to the pitted appearance of the soles It is also known as *haja* which means a sodden or eroded condition The disease has been reported in Ceylon and also the Philippine Islands People who walk barefoot during the monsoons or whose occupation necessitates working barefoot on damp earth and manured soil (for example agricultural laborers domestic servants or housewives) are prone to develop this condition[12,13]

(d) *Tinea versicolor* It is generally held that the fungus caus

actinomycotic lesions and ringworm of hands and feet (athlete s foot) 2 to 4 per cent glacial acetic acid 12 per cent formaldehyde in glycerine 12 per cent resorcin in balsam of peru or solution of aniline dyes in water or 10 to 20 per cent alcohol

For prevention of ringworm of the glabrous skin and foot it is useful to employ a 2 to 4 per cent solution of glacial acetic acid in 10 per cent alcohol and a dusting powder to keep the parts dry [24]

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secondarily infected and others are chronic and lichenified The climatic conditions economic factors and certain habits and occupations also account for difficulty in prevention and cure

The results of clinical trials with some fungicidal drugs in groups of cases of superficial mycosis of the glabrous skin are shown in Table 5 Mercurothiosalicylate (0 05 per cent solution in alcohol and acetone) and phenylmercuric nitrate (1 per cent solution

TABLE 5

CLINICAL TRIALS WITH FUNGICIDAL DRUGS

No of cases	*Fungicidal Drugs*	*Duration of Treatment (weeks) (average)*	*Cured (per cent)*	*Improved to Various Degrees (per cent)*	*No Improvement (per cent)*
100	Synthetic Equivalent of Chrysarobin ½–1% in Lassar s paste	2	84 0	16 0	—
50	Mercurothiosalicylate 0 05% in alcohol and acetone	2	67 0	33 0	—
100	Aniline Dyes brilliant green and gentian violet solution of ½% in 10% alcohol	3	36 0	59 0	5 0
500	Whitfield s ointment	4	28 0	62 0	10 0

further diluted 1 500 in 10 per cent alcohol) were the organic mercurials used The latter was used as a spray on the affected parts and produced satisfactory results among troops in eastern India during World War II

With respect to the aniline dyes it was shown that brilliant green was more effective in vitro against *T violaceum* than crystal violet the latter was more effective against Staphylococcus than the former and a mixture of brilliant green and gentian violet was more effective against *Epidermophyton cruris* but not against *T violaceum* [21 24]

It has been found that the combination of several drugs often works better than a single drug

The following paints are generally used for the treatment of

EDOUARD DROUHET

The Status of Fungus Diseases in France

DERMATOMYCOSES

The dermatomycoses are the commonest mycotic infections seen in France The number of cases of tinea has decreased considerably from the time when Sabouraud began his historic studies on dermatomycoses and their treatment The average of 658 cases observed annually at that time in children in Hôpital St Louis has been reduced to 108 cases per year [1, 2] (See Fig 1) After

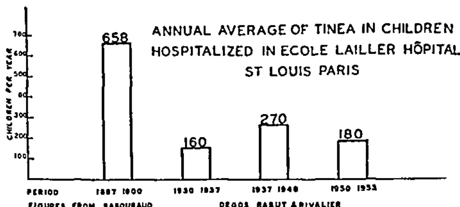


FIGURE 1

World War II a temporary increase was observed which was related to economic conditions and displacement of population Several epidemics of tinea with *Microsporum audouini* were observed in children s homes as well as endemic cases in schools [3]

The percentage of Microsporum remains constant at about 50 per cent (Table 1) but *M audouini* has decreased from 91 to 13 per cent while *M canis* has risen to 86 per cent This is explained by the increased habit of keeping pets in apartments

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TABLE 2

IDENTIFICATION SOURCES AND CLINICAL MANIFESTATIONS OF 259 STRAINS OF *CANDIDA* ISOLATED IN THE LAST THREE YEARS IN THE INSTITUT PASTEUR LABORATORY OF MYCOLOGY (DIRECTOR G SEGRETAIN)

Strains Isolated	*No of Strains*	*Sources of Isolate*									*Clinical Manifestation in Relation with Candida*
		Mouth	*Sputum*	*Stool*	*Gastric Lavage*	*Blood*	*Urine*	*Pus*	*Vagina*	*Skin Nails*	
C albicans	199	45	41	42	14	3	14	5	26	9	15 Systemic Moniliasis with Thrush 29 Moniliasis of Digestive Tract 9 Onychia 2 Black Tongue
C tropicalis	24	6	10		2		2		3	1	2 Bronchopneumonia 1 Black tongue 3 Vaginitis
C pseudotropic	10	3	4		2		1				1 Pneumonia 2 Black Tongue
C krusei (group)	20		4		1		3	2	7	3	1 Subcut Abscess (C parakrusei) 7 Vaginitis
C guillermondi	6		4							2	
Total Cultures	259	54	63	42	19	3	20	7	36	15	

TABLE 1

DISTRIBUTION OF DERMATOPHYTES IN PARIS

1897 1900	1930 - 1937	19 0 1953
MICROSPORUM 50% — M AUDOUINI 91%; M CANIS 9%	MICROSPORUM 51% — M AUDOUINI 75%; M CANIS 25%	MICROSPORUM ,0% — M AUDOUINI 33%; M CANIS 66%
T SCHOENLEINI 10%	T SCHOENLEINI 17 5%	T SCHOENLEINI rlrl F rl 20% ; F 40% ; AF I + 40%
T ENDOTHRIX — T CRATERIFORME; T ACUMINATUM; T VIOLACEUM	T ENDOTHRIX 23% — T CRATERIFORME 53%; T ACUMINATUM 22%; T VIOLACEUM 25%	T ENDOTHRIX 18 8% — T VIOLACEUM 36 %; T SOUDANENSE 5 3%; T TONSURANS 58.4%
	OTHER TRICHOPHYTONS T MENTAGROPHYTES T FAVIFORME	OTHER TRICHOPHYTONS 6.2%

In Paris *Trichophyton schonleinii* has increased slightly from 10 to 23 per cent mostly due to cases of African origin In the endothrix trichophyton group *T violaceum* has taken a more important place having been imported from Mediterranean and Oriental regions

A unique variety of Microsporum was described rather recently under the name of *Sabouraudites (Microsporum) praecox* which rapidly produces numerous elongated fuseaux This condition is *constant and has remained unchanged in the ten years of* observation [9]

The nutritional studies of dermatophytes isolated in France [7] *show vitamin deficiencies in the faviform and tonsurans group* of Trichophyton and a histidine requirement for all strains of *T rosaceum* which have been studied

MONILIASIS

The identification of numerous species of Candida and their relation to clinical manifestations also takes an important place at present in medical mycology in France For the determination of different *species of Candida the well established criteria of classifi*cation by Langeron [32] based on morphology fermentation of sugars and assimilation of carbon and nitrate have been adopted Several technical modifications of these criteria were studied in the

atrics [24, 35] in obstetrics and gynecology [30] in the field of chest diseases in dermatology [11] and in hematology [34] In all clinical manifestations where thrush was found *C albicans* was identified as the species with the closest relation to the clinical aspects of moniliasis A few exceptions were represented by two isolates of *C tropicalis* in bronchomoniliasis one of *C pseudotropicalis* in a case of pneumonia and one of *C parakrusei* in a subcutaneous abscess

Black tongue was relatively rare and was observed only in adults several species of Candida were observed and in two cases *C pseudotropicalis* was identified The latter requires nicotinic acid for its development [27]

A new species of *Candida* was described in a case of canine moniliasis [25]

The treatment with oral nystatin applied in numerous cases of generalized and localized moniliasis showed that this antibiotic has a definite therapeutic activity [24, 26, 29, 31]

The cutaneous allergy to *C albicans* extract was studied in children and showed a high incidence for the first year with a steady increase with age [36]

A hypersensitivity to *C albicans* seemed to be responsible in a case of asthma [37]

ASPERGILLOSIS

Nine cases of well-established aspergillosis were described by Monod Pesle and Segretain [8] The cases had tumorlike lesions due to Aspergillus in the bronchial wall causing a characteristic radiologic image and repeated hemoptysis *A fumigatus* was identified in the majority of these cases more cases of bronchial aspergillosis were recently observed

SPOROTRICHOSIS

This fungus disease once believed to be typical for France and described by Gougerot [43] between 1906 and 1910 in not less than 200 cases has completely disappeared in the last ten years A single observation of sporotrichosis in a dog was recently described [44]

An experimental sporotrichosis with orchitis and gummous lesions of the legs was obtained in hamsters [4] In this experimental

TABLE 3

DISTRIBUTION OF 387 CULTURES ISOLATED FROM SPECIMENS OF HUMAN ORIGIN BETWEEN 1952 AND 1955 IN THE INSTITUT PASTEUR LABORATORY OF MYCOLOGY (DIRECTOR G SEGRETAIN)

Number of Cultures	*Medically Important Fungi*
259	Candida
38	Dermatophytes
37	Geotrichum
34	Aspergillus
6	Nocardia
5	C neoformans
3	Mucor
1	M apiospermum
1	H capsulatum
1	B dermatitidis
1	Hormodendron
1	P hortai

Institut Pasteur and permitted in the last years the rapid identification of a considerable number of Candida[28, 29] (Table 2)

The incidence of Candida in specimens from human origin in the past few years (Table 3) was considerably increased similar to the increase in clinical manifestations of moniliasis Fatal cases of generalized *C albicans* infection occurred in infants[26, 33] Numerous generalized and localized cases were observed chiefly in pedi

in horses Two unpublished cases of human histoplasmosis were observed the first in an engineer who had been in the United States and Syria and while in France developed an acute benign pulmonary histoplasmosis and the second in a man from Bordeaux who had spent most of his life in the French colonies and who developed as the most outstanding clinical manifestation ulcerations of the oral mucosa

Four cases of histoplasmosis were observed in French Africa by Catanei and Kervran [11] and by others [10, 19, 2] In all these cases subcutaneous abscesses were observed and the cultures were identified as *Histoplasma capsulatum* by the presence of the characteristic tuberculate spores however in the tissues the yeast cells were larger than the classical *H capsulatum* A case of fatal hepatic mycosis was observed by Bablet et al [12] but without cultures Van breuseghem described a case in the Belgian Congo He considers the fungus which was isolated from this patient a new species and named it *Histoplasma duboisi* Further studies will be necessary to show whether this thought is justifiable

Recently three African monkeys which were kept in captivity for ten months in the Institut Pasteur in Paris developed spontaneous cutaneous ulcerations with large Histoplasma organisms [14]

An experimental generalized histoplasmosis was obtained in golden hamsters an animal very susceptible to Histoplasma infection [15]

TORULOSIS

Torulosis is not exceptional in France *Cryptococcus neoformans* has been isolated in the Institut Pasteur five times in the last three years In one case isolation was from sputum of a healthy person Ten French cases are on record [4, 4, 47, 48] In one patient a tumor like *form of the sacral region with chronic meningitis was seen* [45] Surgical intervention and treatment with actidione stopped the evolution of paralysis but *C neoformans* was constantly isolated from the spinal fluid In a case of cutaneous torulosis *C neoformans* was isolated from the skin and from the sputum of the patient which is indicative of a pulmonary origin of torulosis

Drouhet and Segretain studied in a mucoid and a smooth variant of *C neoformans* (isolated from a fatal meningitis) the relation between the capsule and virulence Xylose mannose and uronic

infection the various morphologic forms of the organisms were observed in tissue The yeast phase of *Sporotrichum schenckii* was obtained and studied in a synthetic medium containing biotin and thiamin and having a 5 to 10 per cent carbon dioxide tension [41]

SYSTEMIC MYCOSIS

If we consider actinomycosis in the group of fungus diseases then we have to state that it is a very common disease in France Prévot[1] presented a new concept in the systemization of anaerobic Actinomycetes based on the characters of respiration fermentation and other physiologic activities He distinguished in human actinomycosis *Actinobacterium israeli*, *A meyeri*, *A abscessus*, and *A cellulitis*, and in the canine and feline actinomycosis *A baudeti* The strain of *Actinomyces bovis* originally described by Harz was an aerobic Actinomyces

NOCARDIOSIS

A case of this disease with multiple brain abscesses and caused by a variant of *Nocardia asteroides* has been observed [39]

BLASTOMYCOSIS

Blastomycosis produced by *Blastomyces dermatitidis* was described for the first time in a native from Tunis A man twenty five years of age had fatal pulmonary blastomycosis with multiple subcutaneous and osseous abscesses An experimental infection using the culture isolated from this case was reproduced in an African rodent (*Gerbillus hirtipes*) an animal which is very susceptible to the systemic fungi [4]

HISTOPLASMOSIS

A single case of autochthonous histoplasmosis was described in an adult presenting histoplasma like bodies in the mesenteric lymph nodes but unfortunately no cultures were obtained [21] Several surveys in Paris [23] and Bordeaux [18] with histoplasmin showed no specific reactors in human beings Analogous findings were obtained

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acid bound with mannose were identified in the chemical composition of the capsule [49] Segretain and Couteau [50] showed that a tinctorial differentiation is possible between *C neoformans* and the amyloid bodies in the central nervous system

RARE MYCOSES

The first case of rhinosporidiosis was observed in Vietnam in a young native from Pondichéry [40]

Monosporium apiospermum was repeatedly isolated by us from sputum in a case of pulmonary mycetoma

In a case with multiple mycotic brain abscesses a variety of Hormodendrum was isolated which was very close to the form of Cladosporium described by Emmons in similar brain lesions

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toplasmosis and finally coccodioidomycosis and pulmonary pseudotuberculosis caused by *Trichosporon cutaneum* 1 case each

We believe that among the deep mycoses actinomycosis is the commonest but since it is easily diagnosed the internists do not usually send these patients to the Mycological Center Moniliasis of the respiratory tract is frequently reported in our country but *Candida albicans* is considered as a secondary invader in the majority of these cases

Rhinosporidiosis seems to be endemic in the damp zones along the great rivers Parana and Paraguay Fifteen human cases have been reported up to now and about the same number of animal cases in horses mules cows and dogs

South American blastomycosis is endemic in the damp and wooded Chaco region Fifty five cases have been diagnosed in the last twenty years

Coccidioidomycosis (Posadas disease) is also endemic in Argentina in a wide arid zone located between 27 5 and 40 south latitude This area has the same geographic and climatic characteristics as the endemic areas in the United States It is a desert far from the humid winds of the oceans the climate is subtropical and of continental type the soil is sandy and clayish and the vegetation is xerophilic. Half of the clinical cases have been reported from the north end of this area where we have found 20 per cent positive reactors to coccidioidin among rural school children

The first Argentine case of histoplasmosis was described in 1940 and since then 14 new cases have been reported All patients were adults and the majority seem to have acquired the infection in the neighborhood of Buenos Aires The primary lesions were frequently found in the mucous membrane of the oral cavity We have just completed our first endemiologic investigation by using the cutaneous histoplasmin test at a public dispensary for diseases of the respiratory tract and found only 4 positive reactors among 1635 patients These cases were asymptomatic and not associated with pulmonary calcification

PABLO NEGRONI

The Status of Fungus Diseases in Argentina

We have been able to study at the Mycological Center of the School of Medicine of Buenos Aires 1505 cases of superficial mycosis and 73 of deep mycosis during the five years between 1949 and 1953 *Among the superficial mycoses the infections produced by* dermatophytes were the most frequent 1107 We also found 337 infections due to yeastlike fungi and 61 cases of miscellaneous infections (erythrasma pityriasis versicolor etc)

The dermatophytic infections were distributed as follows 615 *cases of ringworm of the scalp 226 cases of ringworm of the gla*brous skin 74 cases of tinea pedis 24 cases of ringworm of the hands and 98 cases of onychomycosis More than two-thirds of the tinea capitis cases were caused by Microsporum We also studied 146 cases of trichophytosis capitis and 11 cases of tinea favosa The *latter is an exotic disease in our country*

Microsporum canis was the commonest agent in the cases of tinea capitis *M gypseum* has been cultured from only one case The most commonly found Trichophyton species were the following in decreasing order of frequency *T violaceum T rubrum, T mentagrophytes T schonleinii* and *T flavus*

The comparatively few cases of tinea pedis reported here are probably due to the fact that dermatologists do not usually require microscopic examination of these patients

The infections caused by yeastlike fungi were distributed as follows *276 mycotic paronychia 40 intertrigo blastomycetica* and 21 mucous membrane infections

The deep mycoses mentioned above had the following distribution 25 cases of actinomycosis 4 cases of maduromycosis caused by Madurella species 30 cases of South American blastomycosis 1 case of European blastomycosis 5 cases of sporotrichosis 6 cases of his

that is potassium iodide However this therapeutic agent is entirely contraindicated in pulmonary tuberculosis No skin involvement has ever been reported in cases of the type described by Magalhaes

In no instance has there been a reliable report of a human case occurring in Brazil of pulmonary mycosis being produced by Aspergillus or Penicillium

A very interesting infection which up to now has always been described as protozoal in origin but which we definitely consider to be a mycosis is that produced by *Pneumocystis carinii* This parasite was first seen by Chagas during his well known studies on American trypanosomiasis He found it in the lungs of guinea pigs which he had inoculated with his *Schizotrypanum cruzi* Led by the then (1909) prevailing views of Schaudinn that there occurred a hemosporidian stage in the life cycle of the trypanosomas Chagas described the parasite as the hemosporidian stage of Schizotrypanum Later Carini and then Arigao both in Brazil reported finding the same parasite in a spontaneous infection in guinea pigs Then the Delanoes in the Pasteur Institute in Paris restudying the question established the new genus and species and gave the parasite its present name For many years *P carinii* has been considered only as a parasitologic curiosity mainly of historical interest and seldom if ever seen by other investigators

It is only recently that several cases have been reported from Central Europe of a peculiar type of severe lung disease found especially in children in which *P carinii* was considered as the only possible etiologic agent We had the opportunity of examining material from these European cases first in the Tropical Diseases Institute in Hamburg and then on slides of the cases submitted by Jirovec to the Sixth International Congress for Microbiology in Rome in 1953 In both instances we were convinced that the parasite must be a fungus and not a protozoan Recently on slides prepared in our laboratory by Dr A S Gabral from the lungs of guinea pigs we again studied the morphology of P carinii and reaffirmed our opinion that it is not a protozoan but a fungus which at least provisionally should be considered as belonging to the Ascomycetes since it displays eight endospores (ascospores) as its only known form of reproduction

The discovery of Pneumocystis in the human lung had already

OLYMPIO DA FONSECA, FILHO

Deep Skin and Pulmonary Mycosis in Brazil

In spite of the fact that the literature on mycotic diseases of Brazil as well as of the Spanish American countries is rather copious many problems relating to these pathologic conditions are still unsolved

We shall not discuss here coccidioidal granuloma which is a well known disease in the United States but is seldom found in other American countries The early reports of its occurrence in Brazil were the result of a tendency to confuse it with the now well known Lutz s disease produced by *Lutziomyces histosporocellulare*

Among the histoplasmoses we shall mention only Darling s histoplasmosis several cases of which have already been reported from Brazil although not enough attention has been paid to its investigation

Pulmonary mycosis produced by *Trichosporon pulmoneum* (*Neogeotrichum brasiliense*) and probably by other related species of fungi as well as by different species of Candida has been fairly commonly observed particularly in the State of Minas Gerais where Octavio de Magalhaes was the first to report its occurrence This disease exemplifies the need for an accurate microbiologic investigation of all cases with a clinical diagnosis of pulmonary tuberculosis in which no acid fast bacilli are found in the sputum Cases are not infrequently found in Brazil in which patients have been unsuccessfully treated for tuberculosis for a period of months before the diagnosis of pulmonary mycosis has been established The correct diagnosis in such cases is particularly important because the disease produced by *T pulmoneum* responds readily to the simplest of all treatments for the deep-seated mycosis

reliable and very confusing indeed As a matter of fact now with a wider experience and a more thorough knowledge of the complexity of the problem in Brazil we believe that only recently and just in one instance we might have been dealing with true American blastomycosis Even in this case however the cultures having not yet developed and the diagnosis having been based only on the clinical picture and on the appearance of the parasites in tissue smears and sections our diagnosis is by no means final With Drs Menandro Tapajós and A E Trindade we are studying the patient who is a young Brazilian male of mixed white and Indian blood living near Manaus in the State of Amazonas He has been suffering for several months with a large collar like ulcero-vegetating and somewhat verrucous lesion on the neck without apparent lung or other visceral involvement We no longer hold the opinion expressed in earlier works of the 1920s about the two Brazilian cases first published by Terra which we then believed could be diagnosed as Gilchrist s disease With no cultures made in these cases and with the skin sections displaying parasites whose morphologic characteristics are somewhat different from those of *Gilchristia dermatitidis* we should actually prefer not to venture a diagnosis

Lutz s Disease Since the so-called Brazilian or South American blastomycosis is not a true blastomycosis we have been calling it Lutz s disease or lutziomycosis the proper and correct name of its parasite being according to the international rules of botanical nomenclature *Lutziomyces histosporocellulare* As for the term neotropical blastomycoid granulomatosis which we formerly adopted it should now be used in the plural and should be applied to a group of diseases not to a single one

The clinical and pathologic features of Lutz s disease are fairly well known but questions related to the etiologic parasite have been subject to much discussion As a matter of fact very little is known about *L histosporocellulare* whose natural saprophytic stage has never been discovered and whose reproductive forms in so far as they have been found in animal tissues and in cultures appear to be the result of abnormal and unfavorable developmental conditions This lack of fundamental knowledge leads to differences of opinion on the systematic position of the parasite

If we are to comply at all with the international rules of botani

been made by Chagas in Brazil but later had been considered as a mistake due to the confusion of slides from man and from guinea pigs Its discovery in Europe as the agent in a new type of pulmonary mycosis calls attention to a problem that probably has been overlooked that of the existence of the same disease in other countries at least in those where guinea pigs have been found to be infected We should add that so far as is known in the guinea pig Pneumocystis infection does not seem to be followed by pathologic changes or clinical symptoms

After mentioning these two types of pulmonary mycosis in which no skin involvement has ever been reported let us consider the several types of what we have called blastomycoid granulomatosis observed in Brazil Since 1938 we have grouped under this name the diseases commonly referred to as the blastomycoses but which are not produced by yeasts (consequently not by Blastomycetes) The budding or yeastlike forms which these agents assume in the diseased tissues should be interpreted as a convergent adaptation leading to similar appearances in organisms not necessarily related The rounded form is probably the result of a process of defense and resistance to unfavorable conditions found by occasional parasites ill adapted to conditions in the host tissues In artificial culture media these fungi usually reassume their normal threadlike forms although occasionally in certain media they can appear as rounded cells

Among the blastomycoid granulomatoses we include at least four different diseases

(1) The so-called American or North American blastomycosis or Gilchrist s disease produced by *Gilchristia (Blastomyces) dermatitidis*

(2) The Brazilian or South American blastomycosis to which we gave the name of Lutz s disease

(3) A mild highly chronic skin mycosis which we called Lobo s disease because its first known case was detected and studied from the clinical point of view by Jorge Lobo in Brazil

(4) Still another skin mycosis similarly benign and very chronic in evolution which has only recently been studied in Brazil

American Blastomycosis So far as Gilchrist s disease or North American blastomycosis is concerned earlier reports from Brazil as well as from several Spanish American countries are far from

reliable and very confusing indeed As a matter of fact now with a wider experience and a more thorough knowledge of the complexity of the problem in Brazil we believe that only recently and just in one instance we might have been dealing with true American blastomycosis Even in this case however the cultures having not yet developed and the diagnosis having been based only on the clinical picture and on the appearance of the parasites in tissue smears and sections our diagnosis is by no means final With Drs Menandro Tapajós and A E Trindade we are studying the patient who is a young Brazilian male of mixed white and Indian blood living near Manaus in the State of Amazonas He has been suffering for several months with a large collar like ulcero-vegetating and somewhat verrucous lesion on the neck without apparent lung or other visceral involvement We no longer hold the opinion expressed in earlier works of the 1920s about the two Brazilian cases first published by Terra which we then believed could be diagnosed as Gilchrist s disease With no cultures made in these cases and with the skin sections displaying parasites whose morphologic characteristics are somewhat different from those of *Gilchristia dermatitidis*, we should actually prefer not to venture a diagnosis

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If we are to comply at all with the international rules of botani

cal nomenclature we cannot go on using the names of *Paracoccidioides* or *Blastomyces brasiliensis* for this fungus The name Blastomyces was applied to a different species of fungus (a mere synonym of *Aleurisma flavissimum* Link) described in 1888 by Costantin and Rollan ten years before being used by Gilchrist and Stokes for the parasite of American blastomycosis So far as the specific name *brasiliensis* is concerned it is based on *Zymonema brasiliensis* which even if it is not a *nomen nudum*, as some people seem to believe is undoubtedly a *nomen confusum* since it was established by Splendore for a mixture of at least two but probably several different fungi whose identification is now impossible But few people bother to read critically Splendore s original paper and the prevailing tendency continues to be that of using a name for the South American parasite that cannot find support in the international rules of nomenclature Of course the genus Paracoccidioides based on the non valid species *brasiliensis*, also has no valid nomenclatural status

One of the most characteristic features of Lutziomyces in the host s tissues is its mode of reproduction After division of the nucleus into a large number of small nuclei the latter migrate to the periphery of the mother cell Then successive layers of the newly formed nuclei together with a very small amount of protoplasm push through the cell wall a part of which is pressed out and becomes the cell membrane of the ectospores so formed In this way a layer of ectospores appears on the surface of the mother cell These spores (some people prefer to refer to them as multiple buds *others call them cryptospores) and successively new* layers of ectospores appear until all the nuclear material contained in the mother cell is exhausted This mode of reproduction was first seen by Lutz and was then well described by Vianna Haberfeld ourselves *and many other authors It can at times be observed in some* artificial media although usually only chlamydospores are to be found As stated in some of our early works depending on the media there may be a complete absence of round cells or they may appear in more or less great numbers

We feel justified in continuing to group as we have done since 1928 the clinical forms of Lutz s disease into lymphatico tegumentary and lymphatic visceral However we should add that quite

often such forms represent only different stages of the development of the same disease

When a purely lymphatic visceral picture develops without any cutaneous oral or tonsillar lesions the disease is easily mistaken for Hodgkins lymphogranuloma or a malignant visceral growth These however are rare occurrences

More frequently the lesions are first noticed either in the skin most commonly on the face or on the oral mucosa or in the tonsils Simultaneously there is an enlargement of the submaxillary cervical or other groups of lymph nodes in the neighborhood of the tegumentary lesions The cutaneous lesions as well as those of the mucosae spread slowly over a period of months New ulcerative or ulcero-vegetating lesions may appear on the skin mainly on the face displaying as in the earlier lesions many minute hemorrhagic points on their surface

In these cases an infiltrative process usually develops in the tissues of the lips cheek and nose This severely reduces facial movements and almost completely suppresses movement of the lower maxilla and also at times gives to the face a more or less elephantiasic appearance Sialorrhea develops simultaneously and a more or less pronounced state of cachexia rapidly ensues

However long before this stage is attained pulmonary lesions may be found in most patients The lung involvement is followed by the clinical picture of pseudotuberculosis with cough thoracic pains and bloody or purulent sputum Roentgenologic examination may reveal either a miliary distribution of the lesions or more or less marked areas of rarefaction leading to the formation of cavities The parasites may be found in the sputum or are ingested and are excreted in the stools Finding of the parasite in the patient s stools however is not pathognomonic of lung involvement not only because it could come from an oral or tonsillar lesion but also because among the most frequent lesions in this disease are the ulcers found in the mucosa of the cecum occasionally in the colon and much more rarely in the rectum

In Lutz s disease as in granuloma coccidioides there may be bone lesions and gummatous lesions of almost any organ

Until the introduction of the sulfa drugs in the treatment of Lutz s disease it was always fatal death inevitably occurring after

a few months or a year or two With the help of sulfathiazole sulfadiazine or the other sulfonamides a clinical cure can now be obtained But since relapses always occur when this treatment is interrupted for a more or less protracted period in the course of several years a patient may receive a few kilograms of one or the other of these sulfa drugs a situation that is now currently seen in Brazil

Purely Cutaneous Blastomycoid Granulomatoses Since 1931 two new and very peculiar types of blastomycoid granulomatoses have been discovered in Brazil Their clinical histopathologic and mycologic features differ markedly both from those of North American blastomycosis or Gilchrist s disease and from those of South American blastomycosis or Lutz s disease These two conditions have in common their chronic and benign character the non ulcerative nodular or tuberous lesions and the fact that they seem to be prevalent in the Amazonian region

The clinical and histopathologic aspects of the first of these diseases have been investigated by Jorge Lobo who sent his patient to us in order to have him studied from the parasitologic point of view *In several instances we consistently succeeded in isolating* from his lesions the same species of fungus which we described under the name of *Glenosporella loboi*

Lobo s patient displayed a number of tuberous more or less keloid like at first somewhat pruritic later slightly painful lesions with no noticeable inflammatory reaction around them All these lesions were localized in the lumbar region and most of them were confluent A clinical diagnosis had been made of tuberous angioma The lesions had begun to develop nineteen years previously at which time the patient lived in the State of Amazonas in the north of Brazil No systemic lesions could be detected by thorough clinical and radiologic examination

From the histologic point of view a characteristic of the lesions in Lobo s disease is the large number of histiocytes

The parasites or their empty cell walls are found in large numbers in the skin and in the subcutaneous tissue in the form of roundish *hyaline cells measuring from 6 to 11 micra* at times up to 14 micra with a double-contoured cell membrane either isolated or geminated or at times in chains or irregular clusters Reproduction is by binary division similar to budding In artificial



FIGURE 1

Patient A P L Lesions on the ear

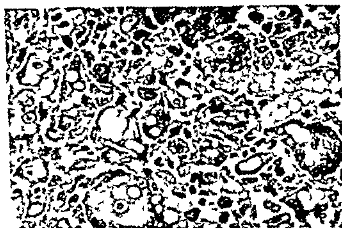


FIGURE 2

Photomicrograph from tissue obtained from A P L demonstrating cell walls of parasites Large number of giant cells

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cultures at room temperature on all usual media it forms white colonies reaching 1 or 1 5 cm in diameter within two to three weeks These colonies are covered with aerial hyphae Microscopically they show a filamentous branched septate hyaline mycelium with at first a few cylindroid arthrospores and later many typical unicellular roundish ellipsoid or ovoid hyaline aleuriospores with a double-contoured cell membrane which reaches 3 5 to 10 0 by 1 5 to 8 0 micra It is difficult to culture the organism directly from pathologic material Subcultures are always easily obtained

Several new cases have been found in Brazil which were described as Lobo s disease However the diagnosis remains doubtful unless confirmed by culture

Unfortunately the original strain of *Glenosporella loboi* was lost and as an obvious result of an error a culture undoubtedly of *Lutziomyces histosporocellulare* mislabeled as *G loboi* was studied from the experimental point of view This error accounts for the conflicting reports found in the literature and for the unacceptable opinion held by the authors of these experimental works that Lobo s disease is just a clinical form of Lutz s disease

The other type of chronic and benign blastomycoid granulomatosis observed in Brazil was first described by us in 1943 The first patient we studied had acquired the disease in the Amazon region twenty years earlier while working as a rubber collector He is still alive after thirty two years with this fungus infection During this time he has been treated in many ways and several attempts have been made to cure him by electrocoagulation and surgical procedures The lesions are localized on the extensor surface of the right forearm and consist of a number of hard nodules firmly adhering to the skin and movable with it The nodules measure from 2 or 3 mm up to 1 5 cm They begin as isolated elementary lesions which under vitro-pressure have an appearance comparable to that of lupomata Over the nodules and around them the skin is more or less tense pinkish or reddish and shining The old nodules may be confluent and show a more or less polycyclical contour or may form masses which attain diameters up to 3 cm Around the nodular lesions there is a wide infiltrated reddish patch which may measure up to 10 cm in diameter On this patch some lupoma like elementary lesions may be seen from time to time The lesions



FIGURE 3

Patient V F S Lesions on the nose



FIGURE 4

Same patient as in Figure 3 Result of incomplete and too conservative surgery with relapse of the lesions around the scar



FIGURE 5

Photomicrograph of tissue obtained from patient V F S demonstrating giant cells and parasites

CTENOSPOROPSIS AMAZONICA TYPE OF PURELY CUTANEO BLASTOMYCOID GRA[illegible]TOSIS

1876 was just the imperfect form of Basidiomycetes of the genus Septobasidium Pat 1892 Also the genus Trichosporium Fries 1849 in which one could also contend that the parasite might be included is not considered more valid since *T fuscum* (Link) which is considered by many as its type species seems to be only the conidial stage of the Pyrenomycete *Rosellinia aquila* (Fr) De N Laccardo Lindau and several other authorities agree in considering Trichosporium as a most doubtful genus As we could refer the parasite to no other known genus we decided to establish a new one Glenosporopsis to replace the no longer accepted genus Glenospora

The description we gave in 1943 for the genus Glenosporopsis is about the same adopted for Glenospora by most authorities and reads as follows

Branched septate hyaline or brownish hyphae sometimes in synuemae Aleuriospores acrogenous or pleurogenous hyaline or brownish in color ovoid or pyriform unicellular with a smooth or slightly corrugated cell wall either isolated or in short chains of two or three

Type species *Glenosporopsis ama onica* O da Fonseca 1943 (Parasitologia Medica vol I 710–711)

Two new cases are now being studied in Manans State of Ama zonas Brazil by Gilberto de Azenedo Teixeira A E Trindade and ourselves

To conclude this paper it seems pertinent to present a brief comment about the classification of the *Fungi imperfecti* that find a place in Vuillemin s (1910) order *Aleuriosporales* (suborder *Aleurioconidiales* Namnizzi) Two different criteria can be adopted According to the first one only two types or genera should be distinguished one mucedinic with hyaline mycelium namely Aleurisma the second dematial with brown or black mycelium Glenospora or as we put it Glenosporopsis According to the other point of view the mucedinic group should be split in differ ent genera and a place could be justified in the classification for Aleurisma Corethropsis Monosporium Gilchristia Histoplasma and Glenosporella

never ulcerate except after and as a result of biopsies and then only temporarily No lesions on any of the mucosae have ever been observed A careful and complete clinical and radiologic examination failed to demonstrate any bone or visceral involvement Examination of the sputum has always been negative for the fungus found in the skin The patient s general health has not been affected

The histopathologic investigation of this case was made by our colleague the late Professor Perma de Azevedo who found a histologic picture characterized by a large number of irregularly arranged giant cells of the Langhans type with many nuclei and an eosinophilic and delicately granular cytoplasm Some macrophages were also found There was almost no lymphocytic or plasmocytic infiltration No necrosis or caseation was seen The histologic picture differs from that of Lutz s disease in which there is severe destruction of the normal structure It differs also from that of Lobo s disease in which there is an intensive macrophagic reaction with few giant cells

The parasites or their empty cell walls are seen in enormous numbers in the skin sections They are roundish hyaline double contoured cells measuring 7 to 12 micra either isolated or grouped in chains of three to six or in irregular clusters They may be extracellular but quite often they are found inside giant cells Artificial culture starting from the lesions is always difficult The first colonies appear in about twelve days and may reach 3 5 cm in diameter The young cultures are white or slightly grayish and covered by a hairy thin layer of aerial hyphae After twenty or thirty days they assume a darker gray or brownish color and their surface becomes powdery Microscopically there is a filamentous branched and septate mycelium which at first is hyaline and then takes on a brown color Reproduction in the cultures is through lateral aleuriospores roundish or ovoid sessile or with a short peduncle with a smooth or slightly corrugated cell wall measuring 4 to 13 micra and also by terminal aleuriospores which are at times ellipsoid or elongated reaching 6 to 19 by 3 5 to 12 micra

These morphologic characteristics would lead one to place this fungus in the genus Glenospora as it is usually understood by medical mycologists that is *sensu* Vuillemin But Couch in 1933 showed that the type species of Glenospora Berkeley and Curtis

Concerning the deep mycoses the greatest Mexican problems are presented by the actinomycotic mycetoma of *Nocardia brasilien sis* [4] and by sporotrichosis [5]

The name mycetoma which connotes the syndrome consisting of tumor fistula and granules is an ancient highly appropriate designation When the granules are formed by an Actinomycete the disease is referred to as an actinomycotic mycetoma when they are formed by a mold it is referred to as a maduromycotic myce toma In other words the presence of granules makes the diagnosis of mycetoma and the structure of these granules determines the type of the disease

Maduromycotic mycetoma is rare in Mexico at least in contrast with the high incidence of actinomycotic mycetoma We have seen cases of maduromycotic mycetoma caused by Cephalosporium Madurella and Monosporium [6]

The anaerobic Actinomycete *A bovis* as well as members of the genera Nocardia and Streptomyces all produce tumors with fistulas and granules *N asteroides* differs from the other Actinomycetes causing deep mycoses in that as well as appearing in the tissues as granules — that is producing actinomycotic mycetoma — it can also be present as short branching filaments and bacillary forms which possess a partial acid fastness In such cases the clinical en tity should be called nocardiosis or pseudotuberculosis There is a tendency to use synonymously the terms actinomycotic myce toma and nocardiosis or to give preference to the latter term for lesions with tumor fistulas and granules This constitutes an error because the name mycetoma has priority and in addition mycetoma can be produced not only by members of the genus Nocardia but also by Streptomyces species such as those designated as *madurae pelletieri* and *paraguayensis* which do not fragment and which upon culture in natural media such as dung and wheat grains do produce conidia [7] Therefore the name nocardiosis should be used only for granulomatous infections of the lung brain and subcutaneous tissues where the etiologic agent *N as teroides* has the morphology of bacilli and short branching fila ments Nocardiosis or pseudotuberculosis is frequent in Mexico Actinomycosis or actinomycotic mycetoma caused by anaerobes is world wide in its distribution It is also frequent in Mexico

The difference between lesions caused by aerobic Actinomycetes

ANTONIO GONZALEZ OCHOA

The Status of Fungus Diseases in Mexico

Due in part to the widely varying biogeographic conditions all the fungus diseases that have ever been described in the cosmopolitan literature are encountered in Mexico with the exception of black piedra

Concerning the dermatophytes their incidence is extremely high and the most striking feature is the preponderance of tinea capitis caused by Trichophyton (90 per cent) compared to that caused by Microsporum (10 per cent) Practically all the trichophytic ringworm of the scalp is caused by *T tonsurans* (94 per cent) and the microsporal by *M canis* (100 per cent) [1] The use of thallium acetate has to a considerable extent solved the therapeutic problem

Infections of the glabrous skin are characterized by the rarity of tinea pedis in rural districts as contrasted with a marked predominance of tinea unguis [2] Generalized tinea corporis caused by *T rubrum* constitutes an unsolved problem with respect to treatment [3] in the tropical regions about the Gulf of Mexico particularly in the States of Veracruz and Tamaulipas In the State of Puebla a subtropical region there exists a focus of tinea *imbricata* which at present is not a public health problem [2]

In some coastal regions on the Pacific as many as 50 per cent of the people suffer from pityriasis versicolor The so-called achromia parasitaria the achromic clinical variety of pityriasis versicolor is also frequent This must be considered in the diagnosis of pinta particularly in zones where both diseases exist such as in the State of Chiapas

Tinea negra is a very rare dermatomycosis which has been observed only in the State of Yucatan

The other superficial mycoses with the exception of black piedra are commonly seen but without any specific peculiarities

three types comprise clinical forms which have a morphologic basis that is in close correlation with the physiopathology of the lesions

The *lymphangitic type* represents the classical picture of the dis ease When the initial lesion occurs on the extremities usually the upper it is referred to as gummatous ascending because the lesions follow almost straight lines along the length of the limbs In the gummatous form affecting other regions (face back abdomen) the nodules do not show arrangement in ascending lines but ap pear irregularly in the vicinity of the primary lesion

TABLE 1

CLASSIFICATION OF CUTANEOUS SPOROTRICHOSIS

CUTANEOUS SPOROTRICHOSIS	Lymphatic type	Gummatous ascending on extremities
		Gummatous in other regions
	Fixed type	Ulcerative
		Verrucous
		Acneiform
		Infiltrated plaques
		Erythematous and scaly patches
	Haematogenous type	Gummatous disseminated over the cutaneous surface

The *fixed type* is characterized by the fact that the fungus when it enters the body and produces the primary lesion remains in situ does not spread and only enlarges locally forming a chancre The clinical forms of the fixed type are ulcerative verrucous acnei form infiltrated plaques and erythematous and scaling patches

The *hematogenous type* has only one clinical form the gumma tous type *disseminated* all over the cutaneous surface It is charac terized by numerous subcutaneous nodules scattered over the en tire body

We have suggested that the systemic sporotrichoses be classified into three types (1) primary the designation being self explana tory pulmonary localization being the most frequent (2) con comitant the primary type being associated with the hematoge nous type of cutaneous sporotrichosis and (3) secondary which follows neglected lymphangitic or fixed types

and those caused by *A bovis* (anaerobic) may be suspected clinically largely by their location Thus cervicofacial and abdominal localizations are due ordinarily to *A bovis*, whereas those on the extremities are caused by Nocardia and Streptomyces species However there are some clinical forms such as the thoracic pulmonary mycetoma which may be produced by either *A bovis* or *N brasiliensis* Here it is not possible to make the etiologic differentiation by symptomatology roentgenologic examination morphology of the granules or biopsy Study of the differentiation of the various types depends on successful culture of the micro organisms

In a recent paper [8] we suggested two means of establishing an etiologic diagnosis in this thoracic pulmonary condition The first means is based on the different habitat of the two Actinomycetes which determines the pathogenesis of the infection In the case of *A bovis* an organism of endogenous origin the infection goes from the lung to the thoracic wall whereas in *N brasiliensis* the source of which is exogenous the infection starts on the thoracic wall and eventually extends into the lung The clinical picture is indistinguishable from that of pulmonary actinomycosis

The second means is based on the acid fastness of the central part of the *N brasiliensis* granule and the absence of this characteristic in the *A bovis* granule

In regard to treatment we have been using diamino diphenyl sulphone since 1947 with good results [9]

The prevalence of sporotrichosis in Mexico is so noticeable that it is not rare to see patients in the streets and in the markets in endemic zones One of the most important sources of infection is packing straw As regards classification of the clinical forms of the disease we consider that the French classification and all those which originate from it are completely inadequate We have proposed [5] that the cutaneous sporotrichoses be divided into three types on the basis of their pathogenesis (Table 1) The classification is based on the evolution which the Sporotrichum follows after its cutaneous entrance on whether or not the fungus spreads from the primary lesion and on whether dissemination takes place through the lymphatics or the blood vessels If the spreading is by way of the lymphatics the *lymphangitic type* results if the fungus remains in situ the *fixed type* is produced and if the dissemination occurs through blood vessels the *hematogenous type* results These

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Sporotrichosis of the mucous membranes may occur on the superficial internal mucosae This condition is governed by the same factors as cutaneous sporotrichosis being of the lymphatic or fixed type the pathogenesis follows the pattern of systemic sporotrichosis

A skin test is now used in Mexico for the diagnosis of this disease The intracutaneous injection of 0 1 cc of a polysaccharide



FIGURE 1

Endemic zones of coccidioidomycosis in Mexico

that we obtained from *Sporotrichum schenckii* elicits a positive skin reaction after twenty four hours [10] This test has been evaluated in hundreds of cases and invariably shows a high specificity

Coccidioidomycosis in Mexico is as important as it is in the United States The only regions where it has significant endemicity are the desert area north and south of the border between these two countries Our endemic zone of coccidioidomycosis encompasses the northern states of Baja California Sonora Chihuahua Coahuila Nuevo Leon and Tamaulipas which are in line west to east with California Arizona New Mexico and Texas (Fig 1) [11]

In addition to our endemic northern states there is in the south the State of Colima in which a high incidence of cutaneous reactors to coccidioidin is present (65 per cent in a rural center) This area is surrounded by states free of coccidioidomycosis

South American blastomycosis has also been discovered in Mexico We found two patients within an interval of six months who lived in the same locality an area where flowers are cultivated and where there was the possibility that gladiola bulbs had been imported from Sao Paulo Brazil [12]

Two months ago in collaboration with Martinez Báez and Reyes [13] we reported the first Mexican case of North American blastomycosis

Two cases of cryptococcosis have been found in Mexico up to the present

The number of patients with chromoblastomycosis that have been seen in Mexico makes us realize that this mycosis is important There is no one zone of prevalence and the cases originate from all over the Republic We started to use diamino-diphenyl sulphone to treat this disease but the number of cases treated to date does not permit us to draw definite conclusions

In regard to the other deep mycoses in Mexico cases of systemic candidiasis aspergillosis rhinosporidiosis [14] and histoplasmosis have been observed Among these it is worthwhile to give some consideration to histoplasmosis Every day we discover more cases by means of the intraperitoneal inoculation of the hamster with the blood of febrile patients [5] Two epidemics of acute histoplasmosis one in seventeen boy scouts who entered a cave [6] the other in four men who visited an abandoned mine [17] have also been recently recorded The highest incidence of cutaneous reactors to histoplasmosis is in the area surrounding the Gulf of Mexico

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SOCORRO AUNARIO SIMUANGCO

The Status of Fungus Diseases in the Philippines

The Philippine Islands being a tropical country have available an abundance of material for fungus studies From reports found in our literature it seems that we are not entirely unaware of the possibility of fungi in the etiology of disease However since it is well known that the systemic mycoses have protean manifestations it is possible that some diseases may have been misdiagnosed Drs Barrera* and Punsalang † mentioned cases that went to the autopsy table and were discovered as due to fungi only after histologic sec tions were seen

In Table 1 we see that mycoses account for 13 94 per cent of all skin diseases seen during the period 1952–1954 Of these 13 9 per cent were superficial and 0 04 per cent were deep The super ficial mycoses are the main mycologic problems encountered in medical practice in the Philippines The five most important of these in the order of incidence are tinea pedis tinea circinata tinea cruris tinea manuum and tinea flava (Table 2)

The earliest report of fungus disease in the Islands was made by Strong in 1906[1] when he described a lesion simulating Delhi boil in a thirty five year old Filipino woman He noted the presence of oval bodies found free and enclosed in endothelial cells which he thought were fungi Meleney[2] in reviewing the cases seen by Strong and another reported by Wade[3] considered the intracellu lar organisms described by them as histoplasma like thus includ ing the Philippines as in the geographic area for histoplasmosis The case of histoplasmosis reported by Mendoza[4] in 1947 proved to be a systemic *Candida albicans* infection on further laboratory

* Chief Pathology Department College of Medicine University of the Philippines
† Senior Clinician San Lazaro Hospital Manila

study by Davis[4] No other cases of histoplasmosis have been reported and the fungus has never been isolated

Melo and Belmonte[5] failed to elicit any positive reaction to histoplasmin among 50 children admitted to the U S T pediatric ward Bocobo and Reyes[6] and Halde and Reyes[4] skin tested 1301 adult Filipinos in the Manila area of whom 3 68 per cent reacted

TABLE 1

INCIDENCE OF FUNGUS DISEASES IN SKIN DISPENSARY PATIENTS * MANILA 1952–1954

	Number of Cases	*Per cent*
Mycoses	3,372	13 94
Superficial	3,361	13 90
Deep	11	04
Other skin diseases	20,807	86 06
Totals	24 179	100 00

* Based on data compiled by the Philippine Dermatological Association.

to histoplasmin Very little significance is attached to this small percentage Thus histoplasmosis if it exists must be rare indeed

In California Filipinos have been shown to be susceptible to coccidioidal granuloma Coccidioidin testing by Halde and Reyes[4] showed no reactors in 824 Filipinos No cases of coccidioidomycosis have been reported in the Philippines indicating that it is probably not present there

Three cases of Madura foot have been reported by Musgave and Clegg[7] Monserrat[8] and Romulo[9] Nocardia species were isolated from the 2 cases in which cultural studies were done

Three other cases were seen by us and will be reported in the future In 2 of these cases there was discharge of yellow granules which on biopsy and staining showed actinomycotic type granules The other case showed only a pinpoint size white granule during

operation which when cultured turned out to be *Penicillium funiculosum*

There are 4 cases of pulmonary nocardiosis in the Philippine literature One case reported by Monserrat[10] was that of a four

TABLE 2

PERCENTAGE DISTRIBUTION OF CASES OF SUPERFICIAL MYCOSES ACCORDING TO TYPE OF LESION (DISPENSARY CASES) MANILA, 1952–1954

Type of Lesion	*Number of Cases*	*Per Cent*
Tinea pedis	958	28 50
Tinea circinata	677	20 14
Tinea cruris	545	16 22
Tinea manuum	530	15 77
Tinea flava	519	15 44
Tinea capitis	59	1 75
Tinea unguium	57	1 69
Tinea nigra	9	0 27
Tinea imbricata	7	0 21
Totals	3 361	100 00

* Based on data compiled by the Philippine Dermatological Association.

month old girl who died of syphilis There were no physical findings referable to the lungs but autopsy showed a lung abscess from which a pure culture of *Nocardia asteroides* was obtained The 3 cases reported by de Santos[11] were those of adults (two males one female) who suffered from subacute pulmonary symptoms The sputum contained granules and mycelial filaments No cultures were reported

There has been one report of rhinosporidiosis[12] This was discovered only on histologic examination of a tissue specimen labeled nasal polyps taken from a seven year old boy from Negros

Sporotrichosis blastomycosis *and cryptococcosis have never been* reported Although the names blastomycosis[13] and cryptococcosis[14] have been used in describing certain cases in the Philippines the cases do not correspond to our present day concept of these diseases

We reported a case of chromoblastomycosis[15] in a fifty eight year old farmer with skin lesions of twenty five years duration He presented an ulcerated tumor cauliflower like growths and a verrucous plaque The fungus isolated was *Hormodendrum compactum* This is only the fourth patient in whom this organism has been discovered The other three were from Puerto Rico Tennessee and California

Moniliasis was first reported in 1924 by Smith[16] when he observed yeasts in the mouth and feces of 8 patients with anemia and the sprue syndrome All his patients were whites with the exception of a male Filipino He isolated *Monilia psilosis* Later investigations have shown that Monilia has no etiologic significance in sprue

Aragon and Halde[17] were able to isolate yeasts from the vagina of pregnant Filipino women with no vaginal symptoms In those cases associated with vaginal pruritis they found abundant growth of *C albicans* and *C tropicalis*

Of the superficial dermatophytes three investigations have been done by Bocobo et al[18, 20] to determine the species in the Philippines (Table 3) In tinea pedis *Trichophyton mentagrophytes* and *T rubrum* are found to be the cause with *T mentagrophytes* predominating by 3 6 per cent compared to *T rubrum* 0 4 per cent In the United States the work of Hopkins[21] and Burke and Bumgarner[2] also show *T mentagrophytes* as the commonest causative organism in tinea pedis

In tinea cruris however, the findings are the reverse of those in tinea pedis *T rubrum* is more commonly found than *T mentagrophytes* (30 per cent compared to 3 per cent) This type of infection by *T rubrum* has a greater tendency to spread to adjacent areas and may even become generalized *Epidermophyton floccosum* has been isolated only once *Since some textbooks*[23] *mention*

TABLE 3

FUNGI ISOLATED FROM DERMATOMYCOSES
MANILA, 1948-1950

Types of Fungi	*Type of Lesion*											
	Tinea pedis		*Tinea cruris*		*Tinea circinata*		*Tinea manuum*		*Tinea unguium*		*Totals*	
	No	*%*	*No*	*%*	*No*	*%*	*No*	*%*	*No*	*%*	*No.*	*%*
Number examined	1,216	100 00	66	100 00	63	100 00	97	100 00	6	-	1 448	100 00
Number positive	49	4 0	24	36 0	20	31 7	-				93	6 42
Tychophyton mentagrophytes	44	3 6	2	3 0	4	6 3					50	3 45
T rubrum	5	0 4	20	30 0	14	22 2					39	2 69
T violaceum			1	1 5	1	1 6					2	0 14
Epidermophyton floccosum			1	1 5							1	0 07
Microsporum gypseum					1	1 6					1	0 07
Number negative	1 167	96 0	42	64 0	43	68 3	97	100 00	6		1,335	93 58

* Based on surveys done by Bocobo and Garcia 1948 1949 [18] Bocobo and Rodil 1949-1950 [19] and Bocobo and Gutierrez 1948-1950.[20]

this organism as the common dermatophyte responsible for tinea cruris this is an interesting observation

In tinea corporis *T rubrum* again is the predominating organism (22 2 per cent) This is in contrast to the findings in the United States where Microsporum especially *M canis* is the commonest cause Bocobo[20] isolated *M gypseum* only once Halde[24] also mentioned that in her investigations in the Philippines the Microsporum infection was uncommon She isolated *M gypseum* from the skin in 3 instances

Bocobo reported no cultures from tinea unguium and tinea manuum although Halde[24] said that the incidence of *T rubrum* infection of the toe and fingernail associated with tinea pedis was commoner than that of *C albicans* infections Nearly the same is true for tinea manuum The over all picture for the dermatophytes shows that *T rubrum* is a constant infecting organism in practically all of the superficial types of mycotic infection

Otomycosis is prevalent *Aspergillus fumigatus*[25] was isolated in the one published case In addition Halde[24] cultured *A niger* and *A clavatus* Her cases were associated with bacterial infection

The etiology of trichomycosis nodosa was studied in 1914 by Schobl[26] who cultured Corynebacterium from the hair We[27] saw white concretions of the scalp hair in school children which we attributed to trichomycosis nodosa We were unable to grow any fungi on Sabouraud s agar only *Staphylococcus aureus* and *S albus* Our concretions showed gram positive coccoid forms

Tinea versicolor is seen frequently and is interesting because of the color characteristic which distinguishes it from the cases seen in temperate climates We see cases with brownish scaly macules but the more prevalent type is the pseudoachromic lesion Various names have been given to the latter such as tinea flava by Castellani and achromia parasitica by Pardo Castello and Dominguez In either case we find that treatment with some photosensitizing agent aside from the parasitic medicament helps in restoring the normal color of the skin

Tinea imbricata is seldom seen in Manila but we had an occasion to visit a Moro village where we found it occurring in almost epidemic form Many families including one with fourteen members were infected with the disease Anthropology books show pictures indicating that the pagan tribes living in the mountains are

also affected We can only attribute the extensive infection in them to their infrequent bathing habits and to the practice of sleeping close together on one mat Some patients affected with this disease who came for consultation at a free dispensary said that in their town many persons had the same disease When we interviewed them they said that it was a common belief that the disease occurs among those who have come in contact with fish washings However since they live along river banks we surmise that the spread of infection can be traced to the habit of using a common stone as abrasive for cleansing of the skin The disease is caused by *T concentricum*, and it had been successfully cultured by Reyes and Halde from cases in the Philippines

Although a photograph labeled Philippine case is in Stitt s *Diagnosis Prevention and Treatment of Tropical Diseases* revised by Strong no literature on tinea imbricata is available in the Philippines The first mention of the presence of this infection there was by the British navigator William Dampier in his *Voyage Round the World* in 1729 [25]

The disease is easily recognized The lesions consist of shingle like papery scales arranged in concentric circles and once seen can hardly be mistaken for any other disease We have seen cases with involvement of the entire body except the palms soles nails and scalp even in children as young as one year old

Although tinea imbricata is considered a superficial skin disease many authorities claim that it is resistant to treatment We have no experience in the treatment of this disease as we were unable to follow up the few cases we saw in the dispensary but I consider this disease interesting enough to warrant further studies From what we have seen in Mindanao it is one of the major skin diseases among the Mohammedan tribes and also possibly the pagan tribes

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A new fungus capable of producing dermatophytosis was identified as *Stachybotris alternans* This fungus was unknown to the medical world until 1937 and its appearance as a cause of skin disease was a complete surprise to physicians as well as to mycologists The axillae the pubes and other intertriginous regions appear to be the sites of predilection in man Here it first appears as erythematous macules which later develop into the characteristic lesions When the genitalia are involved considerable inflammation and swelling result However this is primarily a disease of horses Between 1931 and 1938 this disease caused the death of 30 000 horses in the Ukraine In the horse the disease is manifested by general intoxication and inflammation of the mouth esophagus stomach and kidneys This fungus grows primarily on wet straw from which it passes to horses and man

Otomycosis in the Ukraine is caused by such fungi as *A niger A flavus A fumigatus* and sometimes Penicillium From the ear infection can extend into the brain producing meningitis Dr Poljansky s article on an abscess of the temporal lobe of a man from which he isolated Penicillium is an example of this I have observed a case of stomycosis due to *A niger* in a laboratory technician who worked consistently with this fungus

Fungus infections of the eye are to my knowledge little known in the Ukraine Personally I know of only one case in Kharkov in which *A fumigatus* was isolated from an infected cornea

Dr Tschernyak has described several cases of fungus infection of the stomach and kidney in animals in Russia Identification of the fungus was made by the coloration of the involved organ It is his opinion that fungus infections of the gastrointestinal tract in animals are much more widespread than is commonly assumed

So far as I know there is no published data on fungus infections of the gastrointestinal tract of human beings in the Ukraine However at a conference of the Microbiological Institute of the Academy of Science of the U S S R in 1938 after I gave my report on toxic and pathogenic fungi discussion brought out that during the autopsy of famine victims in 1932 and 1933 many cases of fungus infection of the gastrointestinal tract were observed It was explained by the fact that the hungry population was eating moldy refuse and that the famine weakened individuals were an easy prey for these micro-organisms

V T PANASENKO

The Status of Fungus Diseases in the Ukraine

The fungus diseases of man and animal are widespread in the Ukraine We do not possess factual data concerning the morbidity and mortality of fungus diseases in man since only case reports are recorded in the literature This lack of data in part stems from the fact that few physicians are working in the field of mycology in the Ukraine

Of the various types of fungus disease found in the Ukraine the *dermatomycoses are the most widespread and have been the most* thoroughly studied Dr J S Popov of the Institute of Dermatology in Kharkov has recorded 529 cases of the dermatomycoses observed during a two year period These represented approximately 9 per cent of all skin disease recorded at that institution The commonest causes of these dermatomycoses were species of Trichophyton but other fungi were also encountered *T violaceum* was the most commonly found in the Ukraine followed by *T gypseum T ac cuminatum T crateriforme* and occasionally *Microsporum lano sum*

The scalp extremities nails and buttocks were the usual areas of involvement with these fungi

Besides the indicated species other fungi were isolated but their participation in the pathologic process often remained unex plained The fungi most often received by us from the Institute of Dermatology in Kharkov which were isolated from skin lesions and abscesses were *Aspergillus nidulans A flavus A versicolor, Penicillium lilacinum Scopulariopsis brevicaulis* etc Whether they were the primary causes of the mentioned diseases or second ary invaders was undetermined Dr Leshchinsky reported the iso lation of *A flavus* from an abscess He contended that this organ ism was the sole cause of the abscess

of the significant forces which incite and maintain disease save for the obvious role of the parasite itself When medical students answer that the cause of epidemic tinea capitis is *Microsporum audouini*, such a reply is not wrong but it is certainly inadequate and unsatisfying This type of answer also shows how the germ theory has come to dominate thinking to the extent that the mere presence of the germ is considered a sufficient cause for the incitation of disease A considerable experience shows otherwise

Even when one tries to inoculate the heads of children with great quantities of *M audouini* spores it is found that about 50 per cent of the theoretically susceptible population are resistant Furthermore the infection is trivial and short lasting in many indicating high resistance Considering the intimate play habits of children and the billions of spores shed from infected scalps it seems remarkable that the great majority of urban children escape infection yet rarely in large cities is the incidence greater than 1 per cent Siblings escape far more often than is consistent with the notion that disease is caused by exposure to pathogens Something more than the parasite is required This something is an elusive quality which we call susceptibility an innate factor defying precise definition

It has been claimed that the adult immunity to this type of tinea capitis is due to an increased concentration of antifungal fatty acids in post pubertal sebum a thesis which is exceedingly attractive but one which I believe is without experimental support[2] It rests entirely upon an inference and not a direct biochemical determination As yet in no ringworm disease has it been possible to reduce to biochemical terms the forces which govern resistance or immunity

Modern views force us to focus less attention upon the organism and more upon the qualities of the host as well as upon various ecologic and environmental factors which influence the relationship between the host and the parasite What seems impressive is not the infectiousness of the ringworm fungi but the high natural resistance of the skin of normal human beings

There are many commonplace examples of the high native resistance against dermatophytes Innumerable individuals have chronic infections of the feet and especially of the toenails from which sources a steady shower of organisms contaminate the gla

ALBERT M KLIGMAN

Some Reflections on the Biology of Ringworm Infections

While a good deal is known about the dermatophytes largely through the work of mycologists and although dermatologists have given detailed descriptions of ringworm diseases there has been insufficient attention paid to the area which lies between the mycologists and the dermatologists that is the host parasite relationship or the reciprocal interaction between the parasitized and the parasite The purpose of this communication is to present certain formulations concerning the host parasite relationship in ringworm infections

In the first place the infectiousness and contagiosity of ringworm fungi have been greatly exaggerated This is a consequence of an overly naive view of the way in which organisms produce disease To be sure there can be no ringworm disease without ringworm fungi but their mere presence is not sufficient to establish an infection It is only when the host is susceptible and the environmental circumstances are favorable that the fungus is given an opportunity to become invasive Unfortunately these other forces which contribute to the pathogenesis of disease are not at all obvious for the most part and especially are we unable to analyze susceptibility All that can be said is that some individuals for unknown reasons seem readily able to become infected while others despite adequate exposure are resistant Causation is complex and consists of a network or chain of interacting forces No single element in the network is by itself capable of producing the disease unless the proper combination of other favorable factors exists

This concept can be summed up in an elementary fashion by considering disease to be a result of *predisposing*, *precipitating* and *perpetuating* factors Only rarely can one discern all or most

planation I believe is that the tissue upheaval leads to collapse of the follicle with rupture of the wall and sequestration and fragmentation of fungus infected hairs into the cutis

From time to time in clinical discussions mention is made of dormant ringworm infections a concept which will not bear close analysis if by dormant is meant the persistence of resting or inactive fungus elements in the skin The normal dynamics of skin growth makes such a condition impossible for there is a steady outward stream of keratinized cells which must cause any passive or resting object to be removed in the same way as a drop of India ink is exfoliated To stay in situ the fungus must actively grow against the direction of the epithelial current continuously invading the newly formed keratin Only by establishing such a growth equilibrium with the growth of the skin can the fungus remain in the same relative position

Granted that there are no dormant infections can ringworm infections be asymptomatic that is to say can the fungus grow in the stratum corneum hair or nails without producing disease? We can practically dismiss this possibility in the case of hair or nails since the growth of the fungus disorganizes these structures resulting in some visible damage viz broken-off hairs dystrophic nails In respect to the glabrous skin asymptomatic infections do not appear to exist significantly if at all except in regions such as the palms and soles where the very thickness of the stratum corneum may protect the underlying structures We have never been able to visualize proliferating hyphae in the normal looking glabrous skin even in patients with widespread ringworm lesions

On the other hand occasionally in a definitely minor proportion fungus elements may sometimes be observed between the toes and on the plantar surface of the foot although there may be no visible reaction or disease The epidemiologic significance of such latent infections is inconsequential as it would have to be since even quite active infections are not for the most part highly transmissible

One of the most provocative questions in this field is how ringworm organisms produce inflammation Any clinical reaction such as redness vesiculation edema infiltration scaling etc must be mediated at a distance by some diffusible substance excreted by the fungus for the organism is not actually *in* the tissue which is re

brous skin yet spread to other parts of the skin from these foci is rare indeed Neither do the contacts of such persons acquire ringworm infections as indicated by the resistance of conjugal mates Moreover we have all been perplexed at the extraordinary tendency for certain chronic infections to remain confined to a localized area for years as for instance individuals who have palmar ringworm of one hand but not of the other or who have one infected fingernail but no others

Finally the real *coup d etat* for the belief in the high infectiousness of ringworm fungi is the exceeding difficulty in establishing experimental ringworm infections of the glabrous skin We have been greatly frustrated in attempts to produce actively spreading ringworm lesions conforming to the natural disease by experimental inoculation carried out under a wide variety of circumstances using the entire spectrum of ringworm fungi Again it is evident that something besides the organisms is required although how to measure or recognize that something is a puzzle The individuals in whom experimental inoculations take do not appear palpably different Only rarely can one discern the operation of *some major etiologic force which predisposes to infection viz* ringworm infections in persons with generalized ichthyosis in various endocrinopathies in malignancies etc and even these instances are uncommon As for environmental factors no one will gainsay the enhancing effect of heat and moisture on certain fungus infections of the skin and particularly of the feet

Anatomically the relationship between the host and the parasite in a situation such as the glabrous skin is unique These fungi are not only keratinolytic which enables them to digest the horny protein keratin the building stuff of the stratum corneum hair and *nails but even more important they are necrophilic* that is to say their proliferation is restricted to dead substrates The dermatophytes cannot reproduce themselves or grow in living tissue In the infected hair particularly one can observe the fungus elements stopping abruptly at the zone of nucleated cells just above the matrix, indicating a very definite limitation to invade even partially viable cells When fungus elements are found in the cutis as in Majocchi s granuloma or in kerion Celsus I would strongly dispute the contention of some[3,4] that this shows a capacity for downward growth into the viable layers of the skin The true ex

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acting According to the classical teaching this substance is an allergen to which the tissue is sensitized and the manifestation of ringworm infection of the glabrous skin is therefore essentially an allergic one[8] While such a concept might hold for extremely inflammatory infections it is clearly inapplicable to the so-called noninflammatory ringworm lesions in which the inflammation is mild

It should be noted moreover that the term noninflammatory denotes a difference in degree rather than a total absence of inflammation The tissue reaction is greatly reduced in say *M audouini* ringworm of the skin but there is still obvious redness scaling and an active border Assuming that the host has not through any previous or current circumstances acquired an allergy to the fungus (trichophytin sensitivity) how shall we account for the inflammatory signs of inflammation? I believe that the answer is a very simple one We have demonstrated that the dermatophytes produce exotoxins as well as allergens These toxins are to be sure very mild when compared to let us say tetanus or diphtheria toxin but they are cytotoxic nonetheless The undiluted filtrate of ringworm cultures injected or applied epicutaneously to nonallergic normal skin will produce a primary toxic effect in most individuals In individuals who are allergic such filtrates applied in the same way produce greater inflammatory responses owing to a combination of allergic and toxic influences The cytotoxic agent has also been detected in cultures grown on ground up keratin obtained from calluses so that there is reason to suppose that it can be produced under in vivo circumstances

In short in the case of ringworm lesions of nonallergic skin the ringworm infection is a kind of biologic contact dermatitis of the primary irritancy type in which the irritant is produced by the living organism but evokes the response in a way essentially similar to the epicutaneous application of any irritant chemical On the other hand in violently inflammatory lesions such as kerions allergic mechanisms are doubtless dominant

served the favorable effect of stilbestrol on ringworm of the scalp in humans Foley and Aycock[5] in 1944 observed that a single dose of 40–50 R U of stilbestrol rendered mice highly resistant to the virulent hemolytic streptococci Reiss[6] found in 1949 that *Candida albicans* and *Cryptococcus histolyticus* showed enhanced growth in medium containing steroid

Abernathy et al[7] showed in 1951 that cortisone increased the severity of experimental brucellosis In 1951 Redaelli et al[8] observed that rats injected with cortisone and infected subcutaneously with *Coccidioides immitis* had developed visceral changes Glaser et al[9] and Thomas[10] showed that cortisone increased the severity of streptococcal and pneumococcal infections Kass et al[11] studied the effect of cortisone and ACTH on the development of pneumococcal infection in mice They found that animals injected with cortisone died sooner than the controls Southam et al[12] noted that large doses of cortisone greatly increased the susceptibility of mice to the development of infection with West Nile Ilheus and Bunyamwera viruses Kligman[13] and his co-workers observed that cortisone caused a spread of the cutaneous *Trichophyton mentagrophytes* infections Selye[14] in 1951 summarized his experiments as follows Experiments on the rat show that both cortisone and ACTH given at toxic dose levels can cause fatal systemic infections which are presumably due to otherwise non pathogenic microorganisms such as are normally present in the body This effect of cortisone and ACTH can be completely prevented by suitable doses of purified STH Roth et al[15] in 1952 showed that cortisone increased the severity of infection induced by *C albicans* In 1953 Scherr[16] showed that cortisone shortened the survival time of mice infected with *C albicans* and that STH reversed this effect

Present reports show the effect of various dosages of cortisone on a number of different fungus infections The usual experimental procedure consisted of preinjection of mice with cortisone acetate (Merck) * in 1 1 dilution with isotonic saline The hormone was injected for three successive days (one injection per day) preceding inoculation of the organism The results of these experiments are summarized in Table I

It may be seen that cortisone in dosages of 0 95 to 3 75 mg per

* Our appreciation is extended to Merck Co for the generous supply of Cortone Acetate and Schering Co for Progynon B (Estradiol Benzoate U.S.P) Proluton (Progesterone U.S.P) and Oreton (Testosterone Propionate U.S.P)

ZBIGNIEW T MANKOWSKI

The Influence of Hormonal Conditions on Experimental Fungus Infection

It is becoming evident from the literature that the pathogenic action of micro organisms depends not only on the characteristics of the invading organism but also on the condition of the host For example there is a clinical report dating as far back as 1899 demonstrating beyond doubt the influence of hormonal conditions on the course of mycotic infections Devillers and Renon [1] reported a case of a woman with chronic membranous bronchitis due to infection with *Aspergillus* sp A few days before each menstruation she would have dyspnea and a severe cough The attack would last a few hours and following it she would expel a membrane of greenish color measuring from 3 to 6 cm in length and from 1 to 2 cm in width Microscopic examination showed that the membranes were formed exclusively by mycelium of *Aspergillus* sp In this case there was a chronic membranous bronchitis due to Aspergillus infection which became worse at the time of the increase in the level of estradiol Also the known tendency of favus to disappear at puberty is most probably due to hormonal influences

The widespread use of hormones in treatment of different human pathologic conditions has stimulated many investigators to evaluate hormonal influences on the course of infectious diseases In 1936 Aycock [2] brought attention to the frequency of poliomyelitis during pregnancy Out of ten castrated female monkeys infected with poliomyelitis virus four out of five not treated with estrin developed poliomyelitis while only one out of five estrin treated animals developed the infection Sprunt et al [3] in 1938 reported that the resistance to the vaccinia is increased if the rabbit has been castrated and then given the estrogenic hormone for a period of three weeks before being vaccinated Law [4] in 1943 ob-

injection increased the mortality rate of mice infected with fungi Higher dosages led to no further increase in death rate The most striking results were obtained with those fungus infections which require a relatively long time for development In the case of *C neoformans* all animals treated with cortisone were dead after two weeks although none of the controls died during this period When mice were infected with *Histoplasma capsulatum* the controls survived whereas those treated with cortisone were all dead within a period of seventeen days Cortisone had a similar effect on the course of infection when the inoculation was performed with a suspension of fungi in 5 per cent mucin

The effects of low dosages of cortisone ranging from 0 0005 to 0 1 mg were also tested In these low-dosage experiments no significant action of cortisone was observed However in one experiment a dose of 0 1 mg of cortisone seemed to have a slight inhibiting effect on the infection

Blood cultures on Sabouraud s agar (0 05 ml of blood was withdrawn from the tail vein of each mouse) showed that the number of colonies was greater in cultures of cortisone treated mice than it was in cultures from mice not treated with cortisone (Fig 1)

Detailed results of white-cell counts in bacterial infections under the influence of ACTH have already been published by Kass et al [17] Investigation of white-cell counts on fungus infections under similar conditions appeared to be of interest. Several counts during the course of each experiment under standard conditions were performed Cortisone in doses above 0 9 mg consistently depressed the number of white cells and produced eosinopenia smaller doses had no such effect and in some cases leukocytosis was observed Autopsies were performed in all cases It was found that the livers of cortisone treated animals were significantly heavier in comparison with those of the controls The spleens of the cortisone-treated mice were markedly reduced in weight in comparison with the controls The adrenals were diminished in weight The organs were immersed in Allen s solution imbedded in paraffin sectioned and stained both with hematoxylin and eosin and with the McManus technique No differences between treated and untreated animals were found The examination of McManus stained slides showed wide variation in number and size of fungus colonies in cortisone treated and control animals alike In sporadic cases

TABLE 1

EFFECT OF CORTISONE ON THE PATHOGENICITY OF FUNGI

Organism	Cortisone: mg per Dose	Cortisone: No of Doses	Cortisone: Length of Injection Period (Days)	Cortisone: No of Preinjections	No of Mice	Day of Death after Inoculation: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	21	22	22
Cryptococcus neoformans, 1:10 000 in	--	---	Control	—	6																		2	1			3
saline, 0 2 ml i v	2 5	6	12	0	6									2	1			1	2								
Penicillium sp 1:100 in 5% mucin	--	---	Control	—	13		2	1			2	1							1						1		5
1 ml i p	3 75	3	3	3	15		10	5																			
Histoplasma capsulatum 1:100 in	--	---	Control	--	10	all survived																					
saline, 0 2 ml i v	2 5	9	17	3	10										1		1		1	2	3	2					
Blastomyces dermatitidis 1:100 in	--	---	Control	--	10								1		4	5											
saline 0 22 ml i v	2 5	4	5	3	10						2	7	1														
Aspergillus sp 1:100 in 5% mucin,	--	---	Control	--	21		7	2	3	1						1		1									6
1 ml i p*	0 94 3 75	3	3	3	30		20	4	4	1																	1
Candida albicans 1:1500 in saline,	--	---	Control	--	30	3	1	1	9	5	8	2		1													
0 2 ml i v *	2 5	3	7-8	3 6	38		13	16	9																		

* Combined experiments Small differences in dosages and frequency of injection or preinj ction had little influ nce on the results

FIGURE 1

Growth of colonies of *Candida albicans* on Sabouraud's agar streaked with blood from untreated and cortisone treated fungus infected animals respectively

among the treated animals the number of colonies in the tissues reached high levels never attained in the controls

Limited experiments performed in order to evaluate the influence of ACTH on the development of experimental aspergillosis showed results similar to those obtained from injections of cortisone

Effects of estradiol on the course of fungi infection were also investigated The results are presented in Table 2 It may be seen that mice infected with *C albicans, H capsulatum* and *C neoformans* which received estradiol have definitely shorter survival time than the controls Among the control mice infected with *H capsulatum* none were dead in a period of twenty days whereas all infected mice treated with estradiol were dead during the same period of time Dosages of 0 025 mg (150 R U) 0 05 mg (300 R U) 0 1 mg (600 R U) and 0 2 mg (1200 R U) of estradiol were used For all these dosages the life span of hormone treated animals was shorter than that of the controls this finding was more conspicuous in females than in males During the experiments white-cell counts were made systematically Results are plotted in the graph shown in Figure 2 Each point represents the average of two counts It may be seen that mice which did not receive injections of hormones responded to inoculation with *C albicans* with an early and high leukocytosis while the hormone treated mice failed to show a similar effect

Animals treated for a prolonged length of time with estradiol frequently showed distention of the urinary bladder A survey of the literature on the subject reveals that Lacassagne [18] was first to observe this effect in mice after prolonged treatment with estradiol He noted that distention of the bladder occurred in females as well as in males Histological examination of the bladder wall showed proliferation of the epithelium of mucosa Similar observations were made by Burrows [19] Murray and Steinkamm [20] observed the same effect of estradiol on the urinary bladder of rats and Raynaud [21] on the bladder of cats (Fig 3)

The mechanism of action of estradiol on the change of course of mycotic infection is unknown It is improbable that the hormone acts directly on the micro-organisms Presumably estradiol acts on the tissue of the host either directly or by unbalancing the usual hormonal relationship

TABLE 2

INFLUENCE OF ESTRADIOL ON EXPERIMENTAL FUNGUS INFECTIONS

	Estradiol			Day of Death after Inoculation																
	Pre-injection	Post-injection	No of mice	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Over 15	Mean Survival	D/SE
C albicans 1:1600	Control		39	0	0	1	1	1	3	8	6	3	3	1	1	1	3	7	11 25± 0 78	
	3X 0 2 mg	5-8 X 0 2 mg	38	5	2	5	2	5	4	4	0	1	2	4	1	0	0	3	7 63± 0 79	3 63± 1.1
Histoplasma capsulatum	Control		10	all survived																
1:100-0 2 I V	3X 0 2 mg	8X 0 2 mg	10									1		5	1	2		1		

				Day of Death after Inoculation														
				8	9	10	11	12	13	14	15	16	17	18	19	20	21	Over 21
Cryptococcus n oformans	Control		20	1				3		4	3			3		1	1	4
1:500-0 2 I V	3X 0 05	5X 0 05	39		1	4	3	16	14									1

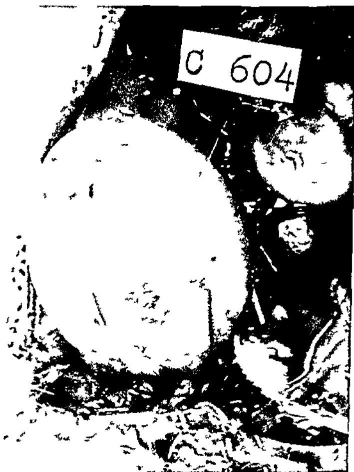


FIGURE 3

Urinary bladder of mouse after prolonged treatment with estradiol

The fact that injections of estradiol enhanced fungus infection in mice suggested that ovariectomy might also affect this type of infection [11] In previous experiments the animals were treated with very high nonphysiologic doses of hormones We expected ovariectomy to have a more conspicuous effect on chronic than on acute infections After several experiments with many different organisms we found that 1 ml of a 5 per cent mucin suspension of *Nocardia mexicana* injected intraperitoneally killed almost all mice during a period of seven to fourteen weeks It was observed that ovariectomized mice lived longer than the controls During a period of fourteen weeks all of the eight infected control mice died but seven out of nine ovariectomized mice survived Injection of estradiol into ovariectomized mice effected a mortality rate similar to that of the control group Additional experiments on a larger scale using ovariectomized animals infected with a more virulent organism that is *C albicans* were performed The results are presented in Table 3 These experiments also showed that the survival time of ovariectomized mice was appreciably longer

TABLE 3

INFLUENCE OF OVARIECTOMY ON EXPERIMENTAL CANDIDIASIS

Organism		# of mice	Day of Death after Inoculation																		Over
			5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	22
Candida albicans 1 1500 in saline 0 2 ml. I P	Control	26	2	2	5	2	3	2	2	1						3					4
	Ovariectomy	16				1		2	3		3		1			1	1	1			13

In the course of initial experiments the animals were kept for several months after ovariectomy before being infected In later experiments the time between ovariectomy and infection was shortened to one week The results of this procedure were similar

The leukocyte counts in ovariectomized uninfected mice were generally higher than in the control mice However the significance of these data is difficult to evaluate because of the well known variability of the white-cell counts in mice

The few experiments performed on castrated males showed only a slight deleterious influence of the castration on the course of experimental fungus infection

The injection of 5 mg of testosterone to a mouse gave much less clear-cut results than treatment with estradiol [22] In experimental aspergillosis the testosterone treated mice had a slightly longer survival time than the controls A mild protective effect of testosterone in candidiasis was also observed

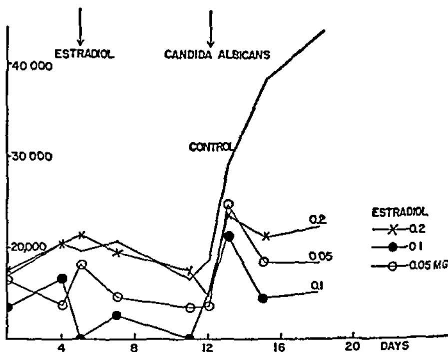


FIGURE 2

Total white cell count of fungus infected mice treated with different doses of estradiol

Progesterone showed no effect on the course of experimental fungus infection when injected alone [22] However it showed remarkable capacity to inhibit the deleterious effect of estradiol on fungus-infected female mice

As both estradiol and cortisone showed the deleterious effect on the development of fungus infection the effect of these hormones given simultaneously has been investigated It was found that these hormones were synergistic in their deleterious effect The fungus-infected mice treated with estradiol and cortisone had a shorter survival time than the mice treated with one of these hormones alone

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and eight 4½ month-old guinea pigs were inoculated in the manner described for Group 1

Group 3 A batch of eight 4½ month-old guinea pigs were inoculated with *Trichophytin Hoechst* in a dilution of 1 2 without oily adjuvants

Group 4 Eight 4½ month-old guinea pigs received no immunizing injection and served as a control

Animals in all groups were infected three weeks after the immunizing injections with a recently isolated strain of *T gypseum* on the right shaved and sandpapered flank This challenging infection did not take place and therefore the left flank was scarified with a blunt scalpel and again infected with the same strain Since the second inoculation failed to produce a uniform infection a third inoculation was made one month after the second unsuccessful challenging infection All animals were inoculated on the right flank with a highly sporulating red pigment producing strain of *T gypseum* * This strain produced the classical clinical manifestation of a *T gypseum* infection which was followed nine weeks later by a reinoculation on the left flank

The results are tabulated in Table 1

The trichophytin test ranged as follows

	Group 1	*Group 2*	*Group 3*	*Group 4*
After 3rd inoculation	0 – 2+	0 – 2+	0 – ±	0 – 1+
After 4th inoculation	0 – 1+	0 – ±	0	0 – 1+

II EXPERIMENT WITH T GYPSEUM IRRADIATED WITH SUPERSONIC RAYS

In a preliminary experiment [2] an alignot fungus suspension of *T gypseum* (Dr Hazen s strain) was placed in a specially made glass balloon and irradiated for six hours with a Siemens ultrasonator Because pertinent data were unavailable as to the optimal oscillation time which would destroy fungi a trial of exposure time varying from one to six hours was instituted The six hour oscillation exposure was found to be the lethal time and was adopted in the preparation of the vaccine In the following experiment cited the supernatant fluid of the irradiated fungus or the irradiated fungus suspension was used

* Courtesy of Dr Elizabeth Hazen New York State Department of Health

FREDERICK REISS and LOUISE LEONARD

Active Immunization against *Trichophyton gypseum* Infection in Guinea Pigs*

Attempts to produce immunity against superficial fungus infections in experimental animals have generally met with failure in the past An exception is the successful active immunization of rabbits against *Trichophyton purpureum* infection [1] This was accomplished with the help of oily adjuvants and killed tubercle bacilli a well known method to enhance antibody production

T purpureum infection however, cannot be reproduced in rabbits with such regularity as *T gypseum* infection in guinea pigs In view of this it was considered feasible to explore the possibilities of active immunization against *T gypseum* infection in guinea pigs in which species *T gypseum* runs a fairly regular and typical course

MATERIALS AND METHODS

I IMMUNIZATION WITH TRICHOPHYTIN

Group 1 *Trichophytin Hoechst* was incorporated in an emulsion composed of Bayol F (paraffin oil) and Arlacel A (mannide mono-oleate) to which dried formalin killed *M butyricum* were added Eight 4½ month old guinea pigs were inoculated intradermally with the above emulsion on ten sites (0 1 ml per site) Each guinea pig received a total of 2 mg of *M butyricum*, 0 5 ml of Trichophytin and 0 5 ml of the Bayol Arlacel combination

Group 2 Broth containing 1 per cent Difco beef extract, 2 per cent Difco dextrose and 0 5 per cent phenol in distilled water served as a medium control for the Trichophytin This liquid was incorporated into the *M butyricum* Bayol F Arlacel A emulsion

* This investigation was aided by a grant DA-47-007-MD-181 from the United States Army

diated *T gypseum* and formalin killed *M butyricum* incorporated in oily adjuvants

Group 5 Three 2½ month-old guinea pigs received saline in an oily suspension of formalin killed *M butyricum*

Group 6 Three uninoculated guinea pigs served as a control for Groups 4 and 5

The challenging infection was performed with Dr Hazen s *T gypseum* strain two and one half weeks after the immunizing in jection

The results are tabulated in Table 2

TABLE 2
MEAN DURATION

	Erythema	*Scaling*	*Crust Format on*	*Alopecia*	*Positive Cultures*
Group 1	26 days	45 days	19 days	47 days	38 days
Group 2	26 days	48 days	21 days	47 days	42 days
Group 3	25 days	47 days	18 days	47 days	42 days
Group 4	25 days	47 days	24 days	45 days	35 days
Group 5	28 days	47 days	15 days	43 days	52 days
Group 6	28 days	45 days	24 days	47 days	41 days

The trichophytin test (eight weeks after infection with *T gypseum*) ranged from 0 to 1+ in all groups with an average of one plus

III REINOCULATION

Since the interval between the immunizing injections and the challenging infection was too short reinfection with the same strain was performed on all animals two weeks after the last nega tive mycological findings Reinoculation was done for the purpose of reinvestigating the possibilities of an existing immunity against *T gypseum* The findings are tabulated in Table 3

The trichophytin test was performed eight weeks after the rein fection with *T gypseum* Due to an enteric disease the test was performed on only 13 surviving animals One animal in group 5 had a 1+ reaction the remaining 12 animals were negative

RESULTS OF IMMUNIZATION WITH TRICHOPHYTIN AGAINST *TRICHOPHYTON GYPSEUM*

TABLE 1

	Group 1	*Group 2*	*Group 3*	*Group 4*
1st Inoculation				
Erythema	0 days	0 days	0 days	0 days
Scaling	18 days	19 days	19 days	19 days
Alopecia	20 days	19 days	19 days	19 days
Positive cultures	0 days	0 days	0 days	0 days
2nd Inoculation				
Erythema	8 days[1]	1 day	2 days	5 days[2]
Scaling	21 days	23 days	24 days	20 days
Alopecia	21 days	23 days	27 days	22 days
Positive cultures	14 days[3]	14 days[4]	14 days[5]	14 days[6]
3rd Inoculation				
Erythema	9 days	12 days	10 days	10 days
Scaling	27 days	27 days	29 days	32 days
Alopecia	33 days	32 days	35 days	31 days
Positive cultures	23 days	21 days[7]	18 days	23 days[8]
4th Inoculation				
Erythema	8 days	9 days	14 days	15 days
Scaling	36 days	32 days	33 days	36 days
Alopecia	40 days	32 days	40 days	32 days
Positive cultures	23 days	26 days	26 days	16 days

(1) Only 2 pigs in this group showed erythema the other 6 pigs were negative.
(2) Only 1 pig showed erythema for 6 days 1 pig for 4 days the other 4 pigs showed no erythema
(3) 2 pigs gave positive cultures for 14 days the other 6 pigs were negative
(4) 2 pigs gave positive cultures for 14 days the other 6 pigs were negative
(5) 4 pigs gave a positive culture for 14 days the other 4 pigs were negative.
(6) 2 pigs gave a positive culture for 14 days the other 4 pigs were negative
(7) One animal in this group had a positive culture for 37 days
(8) One animal in this group had a positive culture for 51 days

Group 1 Eight 2½ month old guinea pigs were inoculated on ten sites (0 1 ml per site) with the above described oily adjuvant and formalin killed *M butyricum* and the supernatant liquid of *T gypseum*

Group 2 Eight 2½ month-old guinea pigs were inoculated with oily adjuvant formalin killed *M butyricum* and saline

Group 3 Five 2½ month old guinea pigs uninoculated served as a control for Groups 1 and 2

Group 4 Five 2½ month-old guinea pigs received six hour irra

that the time interval between the immunizing injection and the challenging infection was not long enough

All these factors have to be taken into consideration and new avenues of active immunization explored

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DISCUSSION AND CONCLUSION

The experiments which are reported here show that immunity against *T gypseum* infection in guinea pigs was not achieved The oscillated supernatant fluid in which washed *T gypseum* culture was suspended contained 28 per cent carbohydrate * and no proteins but apparently the antigenicity of the sonic treated material was not sufficient to produce immunity The irradiated *T gypseum* culture as well as *Trichophytin Hoechst,* both incorporated in oily

TABLE 3

MEAN DURATION

	Erythema	*Scaling*	*Crust Formation*	*Alopecia*	*Positive Cultures* (average)
Group 1	17 days	36 days	9 days	39 days	13* days
Group 2	13 days	35 days	13 days	37 days	9† days
Group 3	19 days	40 days	12 days	42 days	8‡ days
Group 4	16 days	43 days	12 days	45 days	17§ days
Group 5	22 days	35 days	18 days	37 days	11¶ days
Group 6	*14 days*	*43 days*	*13 days*	*46 days*	*16‖* days

The list of figures in the Positive Cultures column cannot be considered as a true mean because of the great discrepancy in the results

In this group fungi were recovered for 6 days in 1 animal 14 days in 2 animals and 23 days in another animal

† In 1 animal in this group fungi were recovered for 27 days for only 6 days in the other 6 animals (1 animal had died previous to the reinoculation)

‡ Fungi were recovered for 14 days in 1 animal for 6 days only in the other 4

§ Fungi were recovered for 27 days in 1 animal 6 days in the other (one died before reinoculation)

¶ Fungi were recovered for 27 days in 1 animal for 6 days in the other 3 animals

‖ Fungi were recovered for 27 days in 1 animal 14 days in 1 animal 6 days in 1 animal

adjuvant with the addition of formalin killed *M butyricum,* also failed to produce immunity

This failure may be due to various factors It is possible that one immunizing injection is not sufficient to produce immunity and furthermore that the carbohydrate content was not enough concentrated in the sonic treated supernatant fluid It is also conceivable

* Grateful acknowledgment is given to Dr C J Umberger Senior Toxicologist and Microanalyst of Bellevue Hospital for his valuable assistance.

A tentative diagnosis of tinea capitis was made on a clinical basis and by use of Wood s lamp This diagnosis was confirmed mycologically by demonstrating the fungus spores in the infected hair in a KOH mount The species of fungus was determined by culturing on Sabouraud s medium

RESULTS AND DISCUSSIONS

Of the 21 cases diagnosed clinically as tinea capitis all were positive by KOH mounts the infected hair showing fungus on the outside in 3 cases and inside the hair shaft in 18 cases Cultures were

TABLE 1
SPECIES OF FUNGI ISOLATED FROM TINEA CAPITIS

Species	*Number Isolated*
T violaceum	9
T tonsurans	6
T megnini	1
M canis	2
M gypseum	1
Unsuccessful cultures	2
Total	21

successful in 19 cases The organisms isolated were the following in order of incidence *Trichophyton violaceum* *T tonsurans* *T megnini* *Microsporum canis* and *M gypseum*

Our results in this study show that Microsporum is not as frequent a cause of tinea capitis as it is in the United States Only 3 of the 21 cases were due to this organism Two were due to *M canis* and 1 to *M gypseum*

In most of our cases the duration of infection varied from one week to five months The longest duration was in *T violaceum* infection which had become widespread in one year The shortest infection one week was found in a case of *T tonsurans* The lesion looked like a circinate ringworm of the skin and the scalp hair did not come out easily on epilation The most rapid spread was due to *M canis* the infection becoming widely scattered after only a month

SOCORRO A SIMUANGCO CARLYN HALDE,
and ALEJANDRO C REYES

A Further Report on Tinea Capitis in the Philippines

Attention was focused on the subject of tinea capitis as a result of our discovery in 1950 of 2 cases of this disease [1] Our interest in this subject was further aroused because of the public health aspect of this infection In the United States this disease constitutes one of the important public health problems because of the frequency with which it occurs in epidemic form [2] Prior to our report in 1954 the only mention of this disease in Filipino medical literature was that by Bocobo and Gutierrez [3] in 1952 who suspected tinea capitis in 7 children seen at the Philippine General Hospital dispensary These cases however had negative findings on mycologic exami nation

In 1955 the Philippine Dermatological Society through a co-operative effort tabulated the incidence of tinea capitis reported from four hospitals in the city of Manila for the period 1952–1954 Fifty nine cases were seen out of a total of 24 179 patients or an incidence of 0 24 per cent The diagnosis was made mostly on a clinical basis Only recently has there been any knowledge of the species of fungus responsible for the disease

METHODS AND MATERIALS

This study covers the period from 1950 to 1955, during which time 21 cases of tinea capitis were seen These cases were discov ered as follows

School survey	3 cases
North General Hospital	5 cases
Philippine General Hospital	3 cases
Private patients	7 cases
Referred by other doctors	3 cases

A tentative diagnosis of tinea capitis was made on a clinical basis and by use of Wood s lamp This diagnosis was confirmed mycologically by demonstrating the fungus spores in the infected hair in a KOH mount The species of fungus was determined by culturing on Sabouraud s medium

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Except for 2 cases which had widespread involvement most in fections were localized The site of greatest predilection was the occiput followed by the parietal parieto-occipital and temple areas There was more evidence of inflammation in *T tonsurans* and Microsporum infections Kerion formation was seen in 4 of 6 cases of *T tonsurans* in 1 case of *M canis* and in the 1 case of *M*

TABLE 2

AGE AND SEX DISTRIBUTION

Age Group (yrs)	*Male*	*Female*	*Total*
0–6	5	1	6
7–12	10	4	14
13 and over	1	—	1
Totals	16	5	21

gypseum This follows observations in the United States that kerion is frequently found in infections with *M canis, M gypseum* and *T tonsurans*[4] We had 2 cases of tinea glabrosa associated with tinea capitis caused by *T tonsurans* and *T megnini* Broken hair stumps the so called black dot ringworm were seen frequently in *T violaceum* infection and were also seen in our case of *T megnini* infection

The ages of the patients varied from four and one half years to thirteen years The greatest number of cases was found in the seven to twelve year group Only 1 case was found in an adolescent There was a preponderance of 16 males to 5 females (a ratio of 3 to 1)

Infection was encountered almost equally in Filipinos and Chi nese In our series there were 10 Chinese (8 males and 2 females) as compared to 11 Filipinos (8 males and 3 females)

A breakdown of the isolated species according to nationality re veals that among Chinese the predominant infecting organism is *T violaceum*, whereas among Filipinos no one organism predomi nated Kurotchkin[6] stated that *T violaceum* is common in North China Bocobo[3] mentioned two Chinese children who took a va cation in China and returned to Manila with a *T violaceum* in fection of the scalp There is a sizable Chinese community in Ma nila totaling 72 376 in a city population of 1 076 240 or 6 72 per

cent In view of the fact that every now and then a fair number of these Chinese go to China for a visit in the homeland there is always the possibility of bringing more of this infection into the Philippines

TABLE 3
INCIDENCE OF SPECIES OF FUNGUS BY NATIONALITY

Species	*Filipinos*		*Chinese*		*Total*
	Male	*Female*	*Male*	*Female*	
T violaceum	0	2	6	1	9
T tonsurans	3	0	2	1	6
T megnini	0	1	0	0	1
M canis	2	0	0	0	2
M gypseum	1	0	0	0	1
Unsuccessful cultures	2	0	0	0	2
Totals	8	3	8	2	21

EPIDEMIOLOGY

In connection with this study surveys were made of schools where our patients were studying and the neighborhood wherein they were residing The school surveys were done on a clinical basis and also by examination of the scalp under Wood s lamp in a darkened room From our experience the Wood s lamp was of little value in detection and follow up of cases because the majority of the cases were caused by Trichophytons Of the Microsporum cases we had the opportunity to examine under the Wood s lamp only the case infected with *M gypseum* The infected hairs within the patch gave a dull greenish fluorescence

Of a total of 6478 school children examined 3617 were Filipinos and 2476 were Chinese In addition 385 children of school age belonging to the pagan tribes were examined Apayaos in northern Luzon 75 Bilaans in southern Mindanao 185 and Moros in Davao 125 Three cases of tinea capitis were discovered among Chinese school children none among the Filipino both Christians and non Christians Each of the 3 cases among the Chinese school children was found in a different school and there was no evidence of spread of infection to immediate contacts

The results of this study also indicate that tinea capitis is commoner in the urban than in the rural areas Only 3 cases came from the rural areas the rest were from the city of Manila

The absence of cases among the pagan tribes may be attributed either to the insufficient opportunity for transfer of the infection due to absence of contact with civilization or to natural immunity to the disease

The socioeconomic conditions likewise seem to play an important role in the epidemiology of this disease Most of the cases of tinea capitis were in children of the low income group who live in congested districts of Manila Five cases in this series however came from the middle income group

In the 2 cases infected with *M canis* there was history of contact with a puppy in one case and with a kitten in the other It was learned from a veterinarian in Manila that they have found clinical fungus infection in dogs aged four to six months However no cultural studies had been done to verify the fungus identification

Spread of infection to one or more members of the family was apparent in 7 instances in our series In the 2 cases of *M canis* infections the disease was transferred to a brother of each of the patients In a case of *T tonsurans* infection a brother of the patient developed tinea circinata In the case of *T megnini* infection the mother developed tinea circinata caused by this organism In a case due to *T tonsurans* infection was spread to a neighbor who also developed tinea capitis due to the same organism

In 2 cases where the hair showed large endothrix spores but whose cultures were unsuccessful the patients were Filipino twins who acquired infection at apparently the same time They attended a Chinese school and there is the possibility that they may have the infection which is common among the Chinese

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CHARLOTTE C CAMPBELL, LAYNE E CARSON, and WALTER H MOURSUND

Tinea Pedis in United States Army Troops Stationed in Puerto Rico and the Comparative Effectiveness of an Antihistamine (Diphenylpyraline) and Undecylenic Acid in Its Treatment

This report is concerned with the extremely high prevalence of tinea pedis in United States Army troops stationed in a subtropical climate the mycotic flora involved and a comparison of the effectiveness of an antihistamine and undecylenic acid in its treatment The latter phase was not completed because of emergency military measures

The study was carried out on 466 infantrymen who were examined immediately after a period of active field training near San Juan Puerto Rico The group consisted primarily of persons native to the island and in many tinea pedis probably had been present since childhood

PREVALENCE OF INFECTION

Following clinical inspection of each individual skin scrapings were removed from infected areas of both feet and sent to the laboratory for study The latter included a three week period of culture at 30 to 32°C on Littman s Oxgall agar (Difco) containing 40μ/ml streptomycin and direct microscopic examination with 10 per cent potassium hydroxide Isolates suspected of being dermatophytes were transferred to Sabouraud s or potato dextrose agar for definitive identification Skin scrapings which did not yield der

matophytes after 14 days were cultured a second time on the Ox gall medium and held for an additional twenty-eight days before being considered negative Finally specimens that were culturally negative following both isolation attempts were examined microscopically for branching filaments

The data shown in Table 1 reveal that 445 of the 466 persons (95 5 per cent) had one or more infection sites on the feet Of these infections were generalized on both feet in 72 1 per cent

TABLE 1

CLINICAL EVALUATION OF TINEA PEDIS AMONG 466 UNITED STATES ARMY PERSONNEL IN PUERTO RICO

	No	%
Positive	445	95 5
Generalized both feet	321	72 1
Localized between toes	101	22 7
Localized other	23	5 2
Negative	21	4 5
Degree of infection		
Scaling only	199	44 7
Scaling plus maceration	177	39 8
Scaling plus maceration plus vesicles	66	14 8
Secondary bacterial infection	3	

were localized between the toes in 22 7 per cent Infection sites in 5 2 per cent were confined to discrete areas on the heels insteps plantar surfaces etc As to the degree of infection in the 445 clinically positive persons scaling only was noted in 199 (44 7 per cent) scaling with maceration in 177 (39 8 per cent) and vesicles in addition to maceration and scaling in 66 (14 8 per cent) There was little evidence of secondary bacterial invasion

This high percentage of clinically apparent infection was confirmed by laboratory study as illustrated in Table 2 Cultural and microscopic studies on specimens from 439 individuals disclosed that 406 (92 5 per cent) were positive by one or both of these examinations Dermatophytes were isolated from 303 persons (69 per cent) and filaments were demonstrated in 103 additional cases in which a dermatophyte was not isolated (23 5 per cent) Specimens from 33 men (7 5 per cent) were negative

TABLE 2

INCIDENCE OF TINEA PEDIS IN UNITED STATES ARMY TROOPS IN PUERTO RICO AS DETERMINED BY MICROSCOPIC EXAMINATION AND CULTURAL STUDY OF SKIN SPECIMENS

	No	%
Positive by culture	303	69 0
Negative by culture Positive by microscopic examination	103	23 5
Negative by culture Negative by microscopic examination	33	7 5
Totals	439	100

TABLE 3

DISTRIBUTION OF 326 DERMATOPHYTIC STRAINS ISOLATED FROM TINEA PEDIS INFECTIONS SEEN AMONG UNITED STATES ARMY PERSONNEL IN PUERTO RICO

Organism	No Cases, Only Isolate	No Cases, One of Two Isolates	Total
Trichophyton rubrum	108	19	127
Trichophyton mentagrophytes	86	11	97
Epidermophyton floccosum	86	16	102
Combinations in dual infections:			
T rubrum and E floccosum		12	
T mentagrophytes and E floccosum		4	
T mentagrophytes and T rubrum		7	

As shown in Table 3 326 dermatophyte strains were recovered Of these 127 (39 per cent) were identified as *Trichophyton rubrum*, 97 (30 per cent) as *T mentagrophytes*, and 102 (31 per cent) as *Epidermophyton floccosum* Two organisms were isolated from each of 23 persons

THERAPY

In earlier studies certain antihistamines were found to possess considerable inhibitory activity against growth in vitro of patho-

genic fungi especially those species which are involved in infec tions of the hair skin and nails When diphenylpyraline (Nopco), the most inhibitory of the antihistamines used in earlier studies failed to produce reactions in 200 volunteers following repeated topical applications of a 5 per cent concentration it appeared to be sufficiently free of sensitizing and irritant properties for trial in these cases It was reasoned that such a compound in addition to a possible fungistatic activity in vivo would also alleviate pruritus and other allergic manifestations frequently associated with such infections

The primary object for the second phase of the study therefore was to compare the therapeutic efficacy of this antihistamine with that of undecylenic acid which is used extensively for the treat ment of dermatomycoses in Army personnel For this purpose each of the two drugs was mixed in a vanishing cream base at a final concentration of 5 per cent Following removal of the first skin specimen each man in the study was given an unlabeled jar of ointment and instructed to apply it to all parts of both feet once daily Men in Group I were issued base without drug those in Group II base containing undecylenic acid and those in Group III base with diphenylpyraline All degrees of infection were present in each group

The initial plan called for a four week period of treatment but for reasons noted earlier the experiment had to be terminated at the end of a two week period Final skin specimens moreover could be obtained from only a limited number of men in each group Clinical inspection of necessity was also cursory Thus the results of therapeutic studies are based on 124 selected subjects *viz* persons whose skin scrapings were positive by culture before treatment and from whom it was possible to obtain specimens at the end of the two week period

The results of these cultural studies are summarized in Table 4 A substantial reduction in the total number of isolates was ob served in both treated groups (II and III) while in the control group (I) almost as many organisms were recovered at the end of the two-week period as before In addition the number of indi vidual species isolated in each of the treated groups appeared to be reduced

Statistical evaluation of these data by chi square analysis was

TABLE 4

NUMBER OF ISOLATES OF SPECIFIC MYCOTIC AGENTS BEFORE AND AFTER A TWO-WEEK PERIOD OF THERAPY WITH DIPHENYLPYRALINE OR UNDECYLENIC ACID

Group	Drug	Total		Organisms isolated:							
				T mentagrophytes		T rubrum		E floccosum		Dual Infections	
		B*	A**	B	A	B	A	B	A	B	A
I	Vanishing cream base without drug (Controls)	36	28	6	3	20	15	10	10	2	4
II	Undecylenic acid	41	12	14	3	13	2	14	7	2	0
III	Diphenypyraline	58	26	-5	15	17	4	16	7	7	2

* Before treatment

** After treatment

also carried out separately for each treatment by causative agent On the basis of this analysis diphenylpyraline was found to be as effective as undecylenic acid in treating tinea pedis caused by *T rubrum* or *E floccosum* On the other hand comparison of the available data on *T mentagrophytes* infections failed to indicate that either drug was more effective than the placebo

No evidence of sensitivity to either drug was observed during this short course of therapy

Since this study was carried out the antihistamines in general have been almost universally discredited as possible therapeutic agents for the superficial mycotic infections primarily because of their sensitizing properties The results in this limited trial however indicate that at least one antihistamine is neither more sensitizing nor less effective therapeutically in the treatment of tinea pedis than a drug that is already widely employed for this purpose

PABLO NEGRONI

Fungi of the Human Bile

We have carried out a mycologic investigation of the bile of 20 patients suffering from various conditions of the biliary tract and duodenum and the bile of a normal person We have studied separately four types of bile from the cystic and hepatic bile ducts (type A) from the gall bladder (type B) from the liver (type C) and from the duodenum (type D) They were collected aseptically after instructing the patient to wash his mouth thoroughly with sterile water

Each sample was centrifuged immediately after collection and a drop of the sediment was examined under a high power microscope The remainder of the sediment was treated with a sterile solution of 10 per cent citric acid for twenty four hours to eliminate bacterial contamination and then cultured on Sabouraud s honey agar in petri dishes After two to six days incubation at 28 and 37 C the various colonies were isolated and studied for species identification We have found that bile contains yeastlike fungi D and A types of bile gave about the same yield of positive cultures (13 of 18 specimens of the D type and 14 of 20 of the A type) We obtained positive cultures of yeastlike fungi from 8 of 14 samples of the B type and from 6 of 18 samples of the C type

These fungi were classified as follows *Candida albicans* 26 strains *C solani* 8 strains *Torulopsis famata* 4 strains *C stellatoidea* 1 strain and *Saccharomyces rouxii* var *polymorphus* 1 strain We seldom checked the following common contaminants *Aspergillus flavus* Pullularia Hormodendrum and Streptomyces

We do not know the role of the yeastlike fungi in pathologic conditions of the biliary tract but we believe that they play a role similar to those found in the feces respiratory tract oral cavity and other natural cavities

From our own experience and from the reports of other authors

we can quote the following facts (1) 4 to 24 per cent of normal persons harbor yeastlike fungi in the mouth and pharynx *C albicans* being the most frequently cultured (2) 19 6 to 30 per cent of sputum samples from patients suffering from tuberculosis cancer of the lungs and other conditions of the respiratory tract yield positive cultures of yeastlike fungi Again *C albicans* is most commonly found (3) 36 of 50 specimens of feces examined by us in 1940 gave positive cultures for yeastlike fungi belonging to the following species in decreasing order of frequency *C krusei*, *C parakrusei* *C albicans* *C zeylanoides*, *C tropicalis*, and *C chalmersi* (4) finally it should be remembered that Candida is a common inhabitant of the vagina We obtained positive cultures in 9 per cent of nonpregnant and 30 per cent of pregnant women *C albicans* was the usual organism cultured in this series

ANNA-MARY CARPENTER

Studies on Candida

Since the use of antibiotics became general several observers have commented on the apparent increase in the number of yeast like organisms isolated from patients This impression is supported by data obtained at the Children s Hospital of Pittsburgh The present study was undertaken with the hope of ascertaining the reason for this phenomenon

One hundred strains of yeastlike fungi were collected from the primary isolation plates of cultures taken from children who were receiving antibiotic therapy Thirty-one per cent of the cultures were from the nose and throat 13 per cent from the urine and 10 per cent from the mouth Other sources were the feces vagina bronchi skin and sputum At autopsy specimens were obtained also from the lung and heart The system of identification was that of Martin et al (J Bact 34 99 1937) Fermentation reactions were controlled by using in addition the medium suggested by Wickerham (Tech Bull 1029 U S Dept of Agriculture May 1951 p 14) Morphology was studied also by the Dalmau cornmeal slide (Ann de Parasitol 7 536 1929) and the tissue culture tech nique of Fusillo et al (Am J Clin Path 22 83 1952)

The present findings (Table 1) indicate a species distribution similar to that reported before the era of antibiotic therapy Seventy six per cent of the strains were identified as *Candida albi cans* and *C albicans* var *stellatoidea*, whereas Martin and Jones reported 74 4 per cent from a greater number of cultures (461) *C parakrusei* was not isolated in this series

Of the one hundred strains collected fifty isolates of *C albicans* six of *C parapsilosis* and six of *C tropicalis* were used to test more than a hundred compounds and mixtures reported to possess fun gistatic properties the sensitivity disk technique was employed As controls five species were procured from the Delft collection in

Holland and six from the Duke University collection Results were recorded as no effect (NE) millimeters of inhibition (I) or millimeters of enhancement (E) Figure 1 illustrates the type of reaction seen

The reagents tested were classed as acids and their esters alcohols aldehydes antibiotics antihistaminics detergents dyes halogens heavy metals oxidizing agents phenols sulfonamides and miscellaneous items such as Castellani s paint Burrow s solution and Bactine

TABLE 1

RELATIVE FREQUENCY OF SPECIES OF CANDIDA ISOLATED

	Martin et al	*Children s Hospital*
	%	%
C albicans	74 4	76 0
C tropicalis	6 7	11 0
C krusei	7 8	6 0
C parapsilosis	10 2	6 0
C pseudotropicalis	0 7	-
Ascogenous yeast		1 0

Only four reagents were notably fungistatic sodium caprylate with zones of inhibition from 3 to 17 mm boric acid which is notoriously toxic to tissues gentian violet the stains of which are unsightly and Cresatin which must be used on mucous membranes with caution A number of reagents were erratic having no effect on some strains causing inhibition of others or producing increased areas of growth Cepacol was curious in this regard Among the forty isolated strains of *C albicans* it showed no effect on thirteen strains 1 to 8 mm of inhibition on twelve and 2 to 9 mm of enhancement on fifteen

An unexpected result was the appearance of a pronounced zone

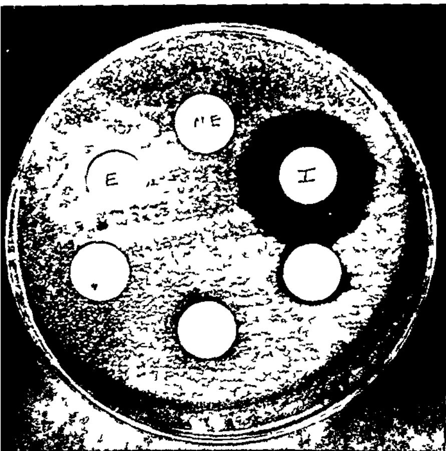


FIGURE 1

Results of testing with sensitivity disk technique
E = enhancement
NE = no effect
I = inhibition

of growth around the sensitivity disk in the experimental series As seen in Table 2 enhancement was evoked with aureomycin paraben in forty nine of fifty strains isolated from patients with

TABLE 2

SENSITIVITY DISK REACTIONS

	50 strains of C albicans isolated from patients			*11 strains used as controls*		
	NE	I	E	NE	I	E
Streptomycin	45	0	5	10	0	1
Penicillin	43	1	6	10	0	1
Terramycin	43	1	6	9	2	0
Aureomycin	47	0	3	8	0	3
Aureomycin-paraben	1	0	49	5	0	6
Sulfathiazole	47	1	2	10	1	0
Benadryl	10	0	40	11	0	0
Sodium caprylate	2	48	0	0	11	0

NE = no effect
I = inhibition
E = enhancement

Benadryl in forty of the fifty strains There was no enhanced growth with Benadryl in the control series

The cause of these differences in strain response was not unequivocally established nor did any factor studied mediate the effectiveness of inhibition by sodium caprylate Noncontributory factors were the source in the patient duration of maintenance in culture size of inoculum or presence of nitrogen phosphate varying species of blood or growth factors in the medium

Contributory factors included preservation in paraffin oil as opposed to lyophilized cultures presence of blood in the medium which was shown previously by Danowski and Tager and occurrence of more than one strain of yeastlike fungus in the sample this was studied from in vivo isolations (Table 3) and by mixing

TABLE 3
REACTIONS OF A MIXED YEAST * CULTURE AND OF ITS COMPONENT SPECIES

	Mixed Culture	*C albicans*	*Hansenula*
Penicillin	0	III	III
Gantrisin	0	4	0
Aureomycin paraben	0	IX	I
Terramycin	0	IV	II
Benadryl	0	VII	0
Boric acid	15	8	14
Cresatin	0	32	5

* Culture isolated from patient

0 = no effect
Arabic numbers = millimeters of inhibition
Roman numerals = millimeters of enhancement

cultures in vitro Strains repeatedly isolated from a patient showed progressive resistance to fungistasis as seen in Table 4 Incubation with radioactive iodine or antibiotics increased the resistance to fungistatic agents

Five strains which had reacted maximally by showing wider zones of enhancement and one stable strain of *C albicans* from the Duke University collection were studied by quantitative tech

TABLE 4

SENSITIVITY DISK REACTIONS ON CULTURES OF *CANDIDA ALBICANS* FROM BRONCHIAL SECRETIONS

	June	*December*	*October*
Aureomycin paraben	VIII	VIII	IX
Benadryl	VII	VII	X
Cepacol	0	III	V
Aureomycin	0	0	II
Gantrisin	0	0	IV
Terramycin	0	0	II
Sodium hyposulfite	0	0	II
Neopenil			III
Boric acid	14	11	8
Cresatin	20	15	10
Sodium caprylate	8	8	7

*Isolated from patient

0 = no effect
Arabic numbers = millimeters of inhibition
Roman numerals = millimeters of enhancement

niques One tenth milliliter of a twenty four hour culture from glucose acid broth was seeded in 5 ml of glucose acid broth to which increments of test solution (aureomycin paraben) had been added Growth curves were plotted at six hour intervals by three techniques turbidity readings Kjeldahl nitrogen and volume of packed cells Inhibition where present was one fourth to one half that in the control enhancement did not exceed twice the control level Variations were pronounced within twelve hours attained a

peak at thirty six hours but were at control levels at forty eight hours

With the techniques employed one can conclude that strains recently isolated from patients showed wider variations among themselves than did the control group there was marked resistance to fungistasis in the experimental series The cause of the phenomenon was not established

G W KEITT and D M BOONE

Experimental Genetic and Nutritional Controls of Pathogenicity of *Venturia inaequalis**

The nature of the reactions between pathogen and host which condition infection and disease development is the central fundamental problem of infectious disease Although this problem has many aspects that differ from one disease to another and from animal to plant disease the basic principles concerned are similar throughout the entire range of infectious disease Plant diseases afford some uniquely favorable materials for researches on these basic principles Indeed the first adequate experimental demonstration and interpretation of the role of a micro-organism in the causation of a disease so far as we know was given in the work of Bénédict Prévost in 1807 on the destructive bunt or smut disease of wheat which is incited by a parasitic fungus

Although great progress has been made in the study of infectious diseases of plants knowledge of the specific biochemical reactions that primarily control the inception of disease is disappointingly meager Perhaps the situation in the field of medicine is not very different In our opinion the chief reason for the slowness of progress in elucidating these critical reactions is that the factors concerned are generally too variable or too complex to be identified and analyzed satisfactorily by the methods that have been available

In the simplest case parasitic disease results from the interac

Grateful acknowledgment is made to the many co workers who have participated in these investigations
These studies were supported in part by the Research Committee of the Graduate School of the University of Wisconsin from funds supplied by the Wisconsin Alumni Research Foundation and in part by grant E–140 (C) from the National Microbiological Institute of the National Institutes of Health Public Health Service and grant G–661 from the National Science Foundation

tions of a parasite and a host each varying under the influences of heredity and environment Knowledge of the nature and extent of variation in both parasite and host is fundamental to the understanding of the nature of their interactions in the processes of infection and therapy Adequate control of variation is essential in most lines of basic experimental work on infectious diseases The understanding and control of variation are especially important in fundamental studies on pathogenicity Strains or biotypes of micro organisms differ in pathogenicity How do such strains originate? How stable are they? What controls their pathogenicity?

The most fruitful approach to the understanding and the control of variability in organisms has been through genetic studies Much attention has been given to genetic studies on the higher plants and animals but until recently comparatively little has been done on the genetics of micro organisms

In recent years great progress has been made in genetic and related studies of micro-organisms The techniques and principles of genetics are now being extended to deal with bacteria and viruses Still more important to the interests of the present symposium great advances have been made in the genetics of fungi Outstanding among these has been the development of so-called biochemical genetics in which genetic controls of specific biochemical reactions furnish keys to fundamental knowledge of life processes These recent advances have made available powerful new tools of genetics and biochemistry which enable new approaches to many fundamental biological problems including some of the most basic problems of infectious disease

Of all the groups of plants and animals perhaps none is so rich as the fungi in forms with favorable adaptations for genetic studies in relation to fundamental biologic problems Many fungi have sexual stages which enable refined genetic experimentation and possess vegetative thalli which can be grown in vitro and studied under a high degree of experimental manipulation and control

Certain nonpathogenic fungi in the genus Neurospora were the subject of a series of brilliant investigations which drew attention to some rare adaptations of this type of fungus for genetic and biochemical studies Mutagenic treatments incited mutations for a wide variety of nutritional deficiencies Correlated studies on the inheritance of the mutant characters and the physiology of the

fungus in vitro established experimentally the genetic control of many fundamental biochemical syntheses The mutant characters generally were inherited as if under single-gene control Thus single-gene control or conditioning of specific biochemical syntheses or steps in synthesis was shown

The aim of the work reported in this paper was to employ a pathogenic fungus in genetic and nutritional studies similar to those made with Neurospora and to correlate the results with studies on pathogenicity In this way it should be possible by genetic and environmental control to manipulate the pathogen experimentally through a wide range of its potential plasticity and to study the effects of known changes on its pathogenicity

We and our co-workers have shown that the apple scab pathogen *Venturia inaequalis* an ascomycete with many similarities to the eight spored Neurosporas has exceptional adaptations for correlated genetic biochemical and pathologic studies on the nature of pathogenicity and related basic problems This pathogen freely infects young vigorous unwounded leaves and fruits and may live for many weeks in intimate association with living host cells It has many biotypes with different pathogenic capabilities in relation to different species or varieties of host It can be cultured throughout its life cycle and cross bred at will in vitro It can be maintained for many years in vitro with satisfactory stability of the characters studied It is haploid throughout the parasitic phase and in its vegetative stage in vitro thus permitting study of effects of a single set of genes The ascus contains the complete progeny stemming from a single miosis with survival of all nuclei in an orderly arrangement which permits tracing the line of nuclear descent by isolating the ascospores in their serial order and studying the derived cultures The vegetative cells are uninucleate and free from important problems of heterocaryosis The fungus responds well to mutagenic agents

Studies on the inheritance of pathogenicity in wild type lines of the fungus laid the necessary foundation for the correlated studies on its genetics nutrition and pathogenicity Only two main types of pathogenic reaction were encountered with monosporic wild type lines of the pathogen namely lesion and fleck (Fig 1) The lesion reaction was typically pathogenic with abundant sporulation of the fungus the fleck reaction was nonpathogenic or slightly

pathogenic with *sporulation lacking or sparse* With a given apple variety the fungus lines were referred to as lesion or fleck according to the reaction incited In lesion × fleck crosses pathogenicity to a given variety ordinarily was conditioned by a single lesion/fleck gene pair which segregated alternatively in the first or the second nuclear division in the ascus Each lesion/fleck gene pair studied conditioned pathogenicity to one group of apple varieties and not to another group The working hypothesis was advanced that critical single gene controlled biochemical reactions commonly condition the pathogenicity of *V inaequalis*

It was concluded that the species *V inaequalis* comprises a great number of biotypes with differential pathogenicity to various apple varieties and Malus species and that a major source of such biotypes in nature is genetic combination

Cultures of the fungus were carried out in vitro for some fifteen *years without evidence of* loss *or gain in pathogenicity*

With this background of information experiments with biochemical mutants were undertaken Although this work is still in process the following progress can be reported at this time

Over one hundred biochemical mutants have been induced by nitrogen mustard or ultraviolet treatments of conidia of a typically pathogenic monoascosporic wild type line of *V inaequalis* and identified as to the end product compound for which there was a deficiency Each biochemical mutant character studied genetically segregated from *normal* in 1 1 ratio indicating single gene control In many cases the approximate map distance of the mutant gene from its centromere was determined The fact that in numerous cases genes at different loci affected the requirement for the same end product metabolite makes possible the study of stepwise biochemical reactions

Some sixty five biochemical mutant lines were tested for pathogenicity The general categories of their nutritional deficiencies were reduced sulfur vitamin nitrogen base and amino acid Mutants which were deficient for reduced sulfur generally showed undiminished pathogenicity Of the vitamin mutants studied those deficient for biotin inositol nicotinic acid or pantothenic acid were as pathogenic as the wild type line those deficient for choline or riboflavin showed greatly reduced pathogenicity None of the mutant lines deficient for nitrogen bases or for amino acids were

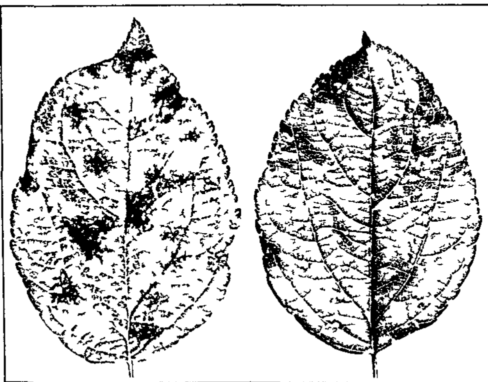


FIGURE 1

Apple leaves inoculated with *V. inaequalis* showing characteristic lesion (left) and fleck (right) reactions

pathogenic although many of these lines incited incipient infection The typical pathogenic reaction was however incited by the mutants deficient respectively for choline riboflavin histidine arginine methionine or uracil when the deficient nutrilite was suitably applied to the leaf surface during the incubation period

This work shows that the pathogenicity of a micro-organism can be reduced or nullified by experimentally induced mutations that the effects of such mutations on the nutrition of the pathogen can be identified in terms of deficiencies for specific metabolites that the mutant characters are unusually favorable material for genetic study and manipulation through crosses made in vitro and that in numerous cases the pathogenicity lost through mutation can be restored by making the deficient metabolite available to the mutant during the incubation period The methods and concepts employed in these investigations seem to afford an approach for further studies on pathogenicity and related basic problems of infectious disease The work is being continued

REFERENCES

It has been impossible in this brief paper to discuss or cite all the work considered The literature dealt with and further discussion of it may readily be found by consulting the following papers

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C E YARWOOD

Therapeutic Action of Sulfur for Powdery Mildews and Rusts

When sulfur is appropriately applied it may cure plants of established infections of powdery mildew (Erysiphaceae) and rusts (Uredinales) and protect them against further infections by these fungi This therapeutic and protective action depends upon such factors as dosage form of sulfur, sensitivity of species age of infection permeation and temperature Therapeutic applications usually require higher dosages of sulfur than protective applications and are therefore more likely to cause injury The practical application of this knowledge to commercial disease control is limited by environment sensitivity of host species to sulfur and economic considerations

There are several alternative chemicals which may be used in place of sulfur but sulfur is still widely used for the control of these diseases Commercial control of powdery mildews is by both therapy and protection whereas commercial control of rusts with sulfur is primarily by protection Therapeutic control of rusts while rather unsuccessful in the past is of greater interest because of past failures and because if practical it would permit delay of fungicide application to a much later date than now necessary[1] Since the infecting rust fungus is inside the leaf therapy is naturally more difficult than with powdery mildews where the mycelium of the fungus is external For the same reason therapy of rusts is more like control of dermatophytes of humans where also the fungus is frequently inside the tissues and control is usually started after infection is established

The outstanding characteristics of sulfur as a commercial fungicide are its volatility its safeness to humans and its economy My remarks will be most concerned with its volatility A simple

method of demonstrating this is to place a test source of sulfur in the bottom of a jar suspend an infected leaf or fungus spores over the sulfur and seal the jar After an appropriate time the leaves or spores are removed and the amount of infection or spore germination is determined [2] Using sodium sulfide as the source of sulfur the effect of sulfur dosage time pH temperature and age of infection are determined Some results are presented in Figures 1

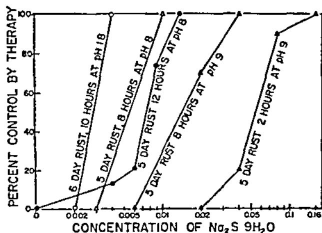


FIGURE 1

The relation of concentration and pH of sodium sulfide solutions to therapy of bean rust by sulfur vapors in closed chambers

and 2 As concentration increased control increased sharply (Fig 1) As time of exposure increased control increased (Fig 2) As pH increased control decreased (Figs 1 and 2) As temperature increased control increased and the temperature coefficient for therapy of bean mildew was about 4 8 [8] As age of infection increased control decreased

If part of the sulfur is radioactive the amount of sulfur absorbed may be quantitatively determined and correlated with the response of the host and the fungus If infected leaves are used it is possible to make autoradiographs of the leaves and relate the accumulation of sulfur to the diseased areas of the leaves [4] and to the efficiency of therapy It is further possible to explore the mechanisms of fungicidal action

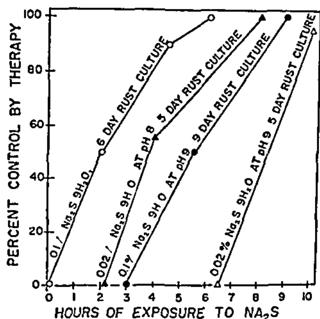


FIGURE 2

The relation of time of exposure and concentration of sodium sulfide solutions to therapy of bean rust in closed chambers

MECHANISM OF FUNGICIDAL ACTION

There are many principles and hypotheses of fungicidal action One is that of selective accumulation [5] According to this the lethal dose of fungicide per unit of tissue may be the same for host and fungus but the fungus accumulates the fungicide in larger amounts than the host and is therefore killed at a lower applied dosage than the host Another principle is that of selective toxicity According to this the amount of fungicide accumulated per unit of tissue may be the same for host and fungus but the fungus is killed at much lower accumulated dosages than the host Either or both of these principles must be involved in all cases of fungicidal action but it may be difficult to determine to what extent each applies These two principles are contrasted in Table I

The role of selective toxicity and selective accumulation was appraised with infections of powdery mildew (*Erysiphe polygoni*) and rust (*Uromyces phaseoli*) on bean (*Phaseolus vulgaris*) Up to eight times as much sulfur accumulated in rusted as in healthy

TABLE 1

SELECTIVE ACCUMULATION VERSUS SELECTIVE TOXICITY IN FUNGICIDAL ACTION—TYPES OF ACTION

Treated Organism	*Amount of Fungicide Accumulated*	*Lethal Dose of Fungicide*	*Result*
Host 1	10 †	100 †	Little or no host injury
Host 2	10	10	Host killed
Fungus 1 on host 1	10	100	No control little or no host injury
Fungus 2 on host 1	10	1	Control by selective toxicity
Fungus 3 on host 1	1000	100	Control by selective accumulation

After arbitrary standard treatment.
† Arbitrary units

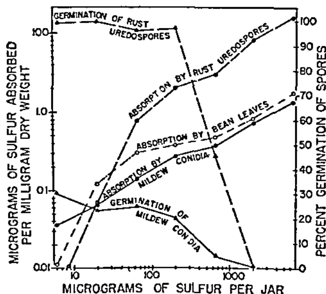


FIGURE 3

The relation of dosage of sulfur to accumulation (absorption) of sulfur by rust spores mildew spores and bean leaves and to germination of rust spores and mildew spores

areas of leaves This naturally suggested that selective accumulation might be responsible for therapy in this case However up to nineteen times as much sulfur accumulated in lesions caused by tobacco mosaic virus in *Nicotiana glutinosa* as in healthy tissue and there was little if any reduction in virus content due to the treatment No selective accumulation was detected in most trials with powdery mildew infections Therefore we would expect selective toxicity to be the mechanism of fungicidal action here

TABLE 2

SELECTIVE ACCUMULATION AND SELECTIVE TOXICITY IN THE FUNGICIDAL ACTION OF SULFUR FOR BEAN POWDERY MILDEW AND BEAN RUST

Treated Organism	*Mg S/g Dry Weight Absorbed from 0 01% Na_2S 9 H_2O at pH 8 8 hrs at 25 C*	*LD_{50} as Mg S/g Dry Weight*	*Result*
Bean leaf	0 4	1 2	No injury
Mildew conidia	0 3	0 1	Selective toxicity
Rust uredospores	2 0	0 5	Selective accumulation

Because of the close morphologic association of host and fungus and the low resolution of the autoradiographs the separate accumulation by host and fungus could not be determined from such trials With healthy leaves powdery mildew conidia and rust uredospores in separate chambers it was possible to measure separate accumulation of sulfur by each Results of two trials with data on spore germination are presented in Figure 3 Accumulation by rust uredospores was much greater than by mildew conidia This supported the idea derived from infected leaves (Table 2) It is tentatively concluded that sulfur therapy of rusts is by selective accumulation and sulfur therapy of powdery mildew is by selective toxicity There are two faults with this type of data however First we do not know how much of the sulfur is external and how much is internal to the fungus although Goldsworthy [6] has demonstrated sulfur inside the germ tubes of peach rust uredospores Secondly we do not know to what extent these separate responses of host and parasite can apply to infected tissues

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ARTHUR C CURTIS

The Aromatic Diamidines, Cinnamic Acids, and Nitrostyrenes in the Treatment of Fungus Diseases

Most of the present knowledge of the pharmacology and clinical use of the *aromatic diamidines — propamidine pentamidine* and stilbamidine — arose from the extensive studies in connection with their activity against protozoa and the treatment of diseases such as trypanosomiasis and leishmaniasis caused by these organisms These data were already available in 1946 when Elson s discovery *of the in vitro antifungal activity of these compounds led to* further in vitro and in vivo animal experiments which justified clinical trials The application of the clinical experiences with these drugs in the treatment of protozoal diseases facilitated their use in the therapy of mycotic infections

*The growing list of cases of systemic mycoses treated with aro*matic diamidines reveals that they are clinically effective against actinomycosis sporotrichosis and particularly North American blastomycosis but not against histoplasmosis cryptococcosis and coccidioidomycosis in man Of the aromatic diamidines stilbami*dine and its derivative* 2 hydroxystilbamidine *have been found to* be the most effective and best suited for parenteral administration Consequently most clinical experience with the aromatic diamidines in mycotic diseases has been with stilbamidine Of 51 reported cases of North American blastomycosis treated with stilbamidine *42 reacted most favorably with disappearance of active* lesions and demonstrable organisms Nine of these cases or 18 per cent of the total were uninfluenced by the therapy or developed relapses and were thus considered as clinical failures

The dosages and mode of administration of stilbamidine have been adequately *described It has been customary to* administer

the drug in daily doses of 150 mg dissolved in 100–1000 ml of 5 per cent glucose solution as a slow intravenous drip in one to three courses of ten to thirty injections each with rest intervals The daily dose may be raised to 300 mg or even to 450 mg on occasional patients The total doses tried in adults ranged from 1 35 to 9 6 gm averaging 3 to 5 gm The patients with recurrent disease have occasioned the administration of relatively large doses of the drug Two such patients have been seen one received 24 5 gm and the other 22 7 gm of stilbamidine isethionate On the other hand blastomycosis has been known to clear up in a child with as little as 0 9 gm of stilbamidine

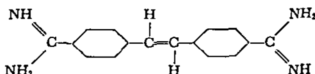
The two commonest toxic manifestations of stilbamidine are the acute nitritoid like crises and the chronic neuropathy over the trigeminal nerve distribution The immediate reactions after intravenous injection of stilbamidine may be alarming consisting of tachycardia breathlessness dizziness nausea vomiting headaches fainting and sweating The present method of administering the drug as a slow intravenous drip has minimized the occurrence of the nitritoid like reactions as well as local thrombophlebitis at the site of injection The development however of the trigeminal neuropathy which consists of sensory disturbances and dissociated anesthesia appearing one to thirteen months after a course of stilbamidine has not been prevented inasmuch as the mechanism of its production is still not definitely known Among 51 cases treated with stilbamidine 27 or 53 per cent developed this peculiar facial neuropathy

In order to obviate the appearance of these undesirable side effects of stilbamidine a closely related compound 2 hydroxystilbamidine has been used clinically in its stead It has the same order of effectiveness as stilbamidine in the test tube against pathogenic fungi and clinical experience with it in the treatment of kala azar multiple myeloma and North American blastomycosis revealed a comparable activity without causing any of the disagreeable trigeminal neuropathy In view of this freedom from such toxic manifestations 2 hydroxystilbamidine can be given in much larger doses and may eventually replace stilbamidine in the treatment of systemic mycotic infections

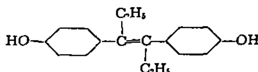
The occurrence of clinical relapses and the development of toxic manifestations with the use of stilbamidine have led to further in

vestigations of other compounds chemically related to it Diethyl stilbestrol by virtue of its stilbene nucleus which is also present in stilbamidine was examined Curtis and Harrell reported two cases of cutaneous North American blastomycosis as successfully treated with diethylstilbestrol pointing out the similarity between its chemical formula and that of stilbamidine In vitro antifungal experiments revealed that diethylstilbestrol had a higher activity than stilbamidine against a greater number of pathogenic fungi The obvious deterrent to the use of diethylstilbestrol clinically as an antifungal agent is its potent hormonal action which precludes its administration in doses high enough to be clinically antifungal

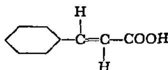
Other groups of compounds related to stilbamidine were also screened for in vitro antifungal activity by means of the serial dilution agar slant method These included cinnamic acid derivatives



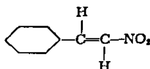
Stilbamidine



Diethylstilbestrol



Cinnamic Acid



β Nitrostyrene

and nitrostyrene compounds The foregoing chemical formulae show their relationships

The cinnamic acid derivatives showed varied and encouraging amounts of activity but the most promising activity was revealed by the nitrostyrenes The nitrostyrenes examined were (1) β nitrostyrene (2) p-methoxy β nitrostyrene (3) p acetoxy β nitrostyrene (4) β methyl β nitrostyrene (5) p methoxy β methyl β nitrostyrene and (6) o-methoxy β methyl β nitrostyrene Compounds (4) and (5) completely inhibited the growth of most of the pathogenic fungi at a concentration of 1 μgm per milliliter of medium The systemic fungi were especially sensitive to their action

These nitrostyrene derivatives were further investigated to evaluate their possible clinical use in fungus infections keeping in mind that the results in in vitro experiments and even in laboratory animal studies do not apply to human cases Very meager data regarding the toxic properties and pharmacologic action of these compounds are available

The antifungal activity of the nitrostyrenes as revealed by the serial dilution agar slant method was confirmed by the filter disk agar plate technique The fungidical nature of this activity was proved by the results of fungicidal experiments The activity of the drug in solution particularly in oily solvents exhibited sufficient stability The drug was found to be available from its ointment form when tested by the agar diffusion plate method

In vitro addition of whole blood decreased the antifungal activity of these drugs This was true for blood obtained from the mouse rat guinea pig rabbit and man This may unfortunately lessen the possible clinical effectiveness of the nitrostyrenes especially with parenteral administration

As a preliminary for animal protection experiments the LD_5 of each compound was determined The values noted in Table 1 were obtained using mice as test animals

Animal protection experiments against infection with *Cryptococcus neoformans Histoplasma capsulatum* and *Blastomyces dermatitidis* failed to disclose any significant protection by β methyl β nitrostyrene or p methoxy β methyl β nitrostyrene except possibly in experiments with *C neoformans* where there appeared to be some degree of protection when two injections of the drug were given one and six days after infection Inasmuch as the

TABLE 1

THE LD_{50} FOR MICE OF β-METHYL-β-NITROSTYRENE AND P-METHOXY-β-METHYL-β-NITROSTYRENE IN VARIOUS SOLVENTS WHEN GIVEN INTRAPERITONEALLY OR SUBCUTANEOUSLY

	β methyl β nitrostyrene	*p methoxy-β methyl β nitrostyrene*
Intraperitoneal, with propylene glycol as carrier	82 mg /kg	130 mg /kg
Intraperitoneal, with gelatin as carrier	232 mg /kg	327 mg /kg
Subcutaneous, with peanut oil as carrier	457 mg /kg	728 mg /kg
Subcutaneous, with Merch vehicle as carrier	480 mg /kg	960 mg /kg
Subcutaneous, with alcohol glycerin as carrier	—	700 mg /kg

nitrostyrenes are water insoluble one of the main problems in these experiments was to find the appropriate solvents for these compounds suitable for parenteral injections

In anticipation of the topical use of the nitrostyrenes in super ficial mycotic infections irritation experiments by percutaneous corneal intradermal and intramuscular routes were performed They revealed a certain amount of primary irritation caused by p methoxy β methyl β nitrostyrene

Taking into account the LD_{50} values the solubilities and the comparative in vitro antifungal effectiveness of the different nitro-styrenes p methoxy β methyl β nitrostyrene was chosen for use in clinical trials being carried out Clinical experience with the drug *is still preliminary but certain significant findings have* emerged even at this early stage

No favorable effect could be claimed with the topical use of p-methoxy β methyl β nitrostyrene in the few cases of *Trichophy*

ton rubrum infection treated One case developed contact sensitization to the drug in ointment form

In the light of the relative resistance of *Candida albicans* to the in vitro action of the nitrostyrenes it was surprising to obtain favorable clinical results in the local treatment of buccal and vaginal candidiasis with p-methoxy β methyl β nitrostyrene This success was significant enough to warrant more extensive clinical trials and to justify the recommendation of its local use in candidiasis of the skin and mucous membranes The drug was used as mouth rinse or vaginal douche in concentrations of 50 to 100 mg in 1 liter of water

The parenteral use of the drug however in the treatment of a patient with recurrent North American blastomycosis was a failure Oral and intramuscular administration did not influence the course of the disease This was at first explained mainly by the nonabsorption of the drug Intravenous administration was accompanied by an initial improvement which however stopped after a week In spite of the failure of the treatment this case was instructive as regards the clinical use of the drug As with the animal experiments the main difficulty encountered was the preparation of the solution for injections inasmuch as the compound is water insoluble The drug was given in concentrations of 50 to 150 mg per liter of 5 per cent glucose solution as a slow intravenous drip This was associated with venospasm and thrombophlebitis which may be explained by the irritative qualities of the drug Aside from these untoward effects no other toxic manifestations were shown by the patient No blood levels could be shown by the agar diffusion technique The reduction of the in vitro activity of the drugs upon mixture with whole blood and certain observations in animals that suggest a combination of these compounds with hemoglobin may possibly explain their inefficacy when given parenterally

DAVID GOTTLIEB

Filipin, an Antibiotic Inhibiting Fungi

Streptomyces filipinensis n sp produces a new antibiotic, filipin [1] * This compound is a broad spectrum antifungal agent which does not inhibit any of the bacteria that were tested The antibiotic is present in both the mycelium and filtrates of the culture medium in which the organisms have grown Media containing oils as a carbon source give greatest yield of antibiotic and the fatty acid components of these oils appear to be the important constituent for optimum synthesis

Filipin can be extracted from the mycelium with ethanol or butanol and can be removed from the culture fluid with ethyl acetate butanol or ether After the solvent is removed the crude material is washed with petroleum ether and then crystallized from chloroform Filipin is soluble in methanol ethanol *n* butanol pyridine dimethyl formamide and glacial acetic acid but is nearly insoluble in water The material is a yellow crystalline solid that exists in two allotropic modifications the first form undergoes a transition to the second form at 147°C and the compound melts at 195 to 205°C with decomposition Analysis of the filipin indicates an empirical formula of $C_{30}H_{50}O_{10}$ molecular weight 571 It is nonaromatic and relatively unsaturated

The ultraviolet spectrum of filipin is characteristic of a polyene [2, 3] with three peaks 355 338 and 322 mu and a shoulder at 305 mu (Fig 1) These data together with a study of the infrared spectrum suggest that it contains isoprenoid groups and is related to the carotenoid series of compounds It has a specific rotation in methanol $\left[\alpha\right]^{22}_{D}$ −148 3 Filipin undergoes autoxidation in air when exposed to light but not when kept under nitrogen This compound can be separated from its nearest relative fungichro-

* Complete reports on this antibiotic are now in press

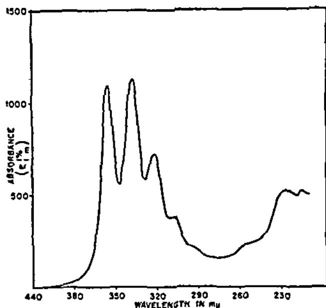


FIGURE 1

min[4] by paper chromatography the infrared absorption and the presence of two allotropic forms

Filipin can be assayed either by the paper disk seeded plate method or by the determination of the amount of absorption of ultraviolet light The biologic method can be carried out with either spores of *Penicillium oxalicum* or with *Saccharomyces pastorianus* for seed The ultraviolet determinations were made with a Beckman spectrophotometer With solid preparation the optical densities at any of the maxima vary directly with the concentration of methanol In culture filtrates however there is a background absorption which interferes with the assay This effect can be nullified by using the difference in absorption between adjacent maxima and minima since the background effect is equal at both wave lengths Such differences are proportional to concentration of filipin A typical absorption curve is given in Figure 2 Solutions of crystalline filipin are used as a standard in both assays

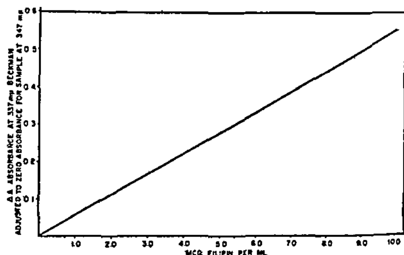


FIGURE 2

BIOLOGIC PROPERTIES

The data in Tables 1 and 2 indicate that a wide variety of fungi are inhibited by filipin including saprophytes plant pathogens and human pathogens *Cryptococcus neoformans* was the most sensitive of the pathogens tested being inhibited by approximately

TABLE 1

MINIMUM INHIBITORY CONCENTRATION OF FILIPIN AGAINST FUNGI PATHOGENIC TO MAN

Test Organism	*μg/ml*
Candida albicans	7 7
Coccidioides immitis	15 0
Cryptococcus neoformans	0 95
Geotrichum sp	31 0
Histoplasma capsulatum	7 7
Hormodendrum compactum	15 5
Microsporum audouini	7 7
Monosporium apiospermum	7 7
Nocardia asteroides	31 0
Phialophora verrucosa	15 5
Sporotrichum schenckii	7 7
Trichophyton rubrum	7 7
Blastomyces dermatitidis	31 0

* Incubation time was 48 hours

TABLE 2

MINIMUM INHIBITORY CONCENTRATION OF FILIPIN AGAINST VARIOUS MICRO-ORGANISMS

Test Organism	*µg/ml*	
	72 hrs	*96 hrs*
Saprophytes and plant pathogens		
Aspergillus niger	3 9	31 0
Colletotrichum lindemuthianum	1 9	3 9
Dendrophoma obscurans	7 8	7 8
Diplodia sp	31 0	1000 0
Endothia parasitica	1 9	1 9
Fusarium oxysporum dianthi	7 8	15 0
Gnomonia fragariae Kleb	0 95	0 95
Helminthosporium sativum	7 8	7 8
Penicillium digitatum	3 9	7 8
Penicillium notatum	31 0	62 0
Phoma betae Fr	3 9	7 8
Phomopsis sp	3 9	7 8
Rhizoctonia solani	500 0	500 0
Sclerotinia sclerotiorum	7 8	15 0
Thielaviopsis sp	3 9	3 9

1 µg per milliliter in an agar dilution test The remainder of the animal pathogens were inhibited by concentrations that varied from approximately 8 to 31 µg per milliliter depending on the species Plant pathogens were inhibited within the same range of concentrations except for *Rhizoctonia solani* which required 500 µg per milliliter for complete inhibition When the cultures were incubated for longer periods of time in the presence of the antibiotic higher concentrations were necessary to prevent growth of the organism Only in the case of *Diplodia* sp was the increase so great that it might affect the use of filipin in any program for disease control

The inhibitory effect of filipin on *Monilia albicans* was due primarily to its fungicidal activity At 50 µg per milliliter death of *M albicans* occurred within a two-hour exposure to the antibiotic Ten µg per milliliter of the compound was also effective but in this case between seven and twenty four hours of exposure were necessary A greater concentration or a longer exposure was necessary to kill spores of *P oxalicum*

Filipin also has antitrichomonal activity Filipin killed *Tricho monas foetus* Riedmuller at a concentration of 50 μg per milli liter markedly inhibited its multiplication at 25 μg and had a delayed effect at concentrations as low at 1 μg

Filipin has very little phytotoxic activity and has been safe even when sprayed at 1000 μg per milliliter It had no phytotoxic ef fect when seeds were soaked in methanolic solutions of 100 μg per milliliter for three hours Such treatment prevented the rotting of pea seeds by fungi In addition sprays of crude filipin reduced the severity of gray leaf spot on tomatoes

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CARLYN HALDE and DONNA NEWSTRAND

The Sensitivity of Pathogenic Actinomycetes to Various Sulfonamide and Sulfone Compounds*

Definite clinical improvement as the result of treatment has established the importance of sulfonamides and sulfones in the therapy of nocardiosis and actinomycosis These drugs have been studied for a number of years and considerable knowledge has been gained concerning structure activity relationship and mechanism of action Although contradictory observations have been made the currently held theory of interference with enzymes necessary for growth is generally used to explain the mechanism of action These drugs by disturbing the utilization of para amino benzoic acid interfere with the action of certain coenzymes essential for bacterial growth The action of sulfonamides is primarily bacteriostatic Recovery from disease caused by sulfonamide-sensitive micro-organisms is believed to be due to the inhibition of growth of the pathogen within the host which thereby provides an opportunity for normal defense mechanisms to destroy the invaders

In the past most in vitro sensitivity tests of Nocardia species and *Actinomyces bovis* have been carried out in complex media containing sulfonamide and sulfone antagonists Although these tests have shown inhibition of growth of the micro-organisms it is believed that media free of antagonists would be more accurate for the determination of the drug most effective against individual pathogens An inhibitor free medium (SR medium base †) has recently become available Originally designed for testing the sul

* This investigation was supported by a research grant No E 786 of the National Microbiological Institute of the National Institutes of Health Public Health Service
† Available from the Difco Laboratories Detroit Michigan

fonamide sensitivity of Group A hemolytic streptococcus, this medium supports growth of Nocardia species and *A bovis* This semi synthetic medium contains casamino acids, amino acids dextrose minerals various growth accessory factors and 0 1 per cent agar Although such a complex medium is not necessary since most species of Nocardia will grow in Czapek s broth and other simple synthetic media growth is considerably heavier and the test is complete within five days to one week

There are two aspects to the present study (1) The sensitivity of thirty four strains of Nocardia species and three strains of *A bovis* to sulfonamides and sulfones was investigated using SR medium (2) Animal studies using infected mice were undertaken to compare the therapeutic effectiveness of diasone and sodium sulfadiazine

PROCEDURES

IN VITRO STUDIES

The dehydrated SR medium was reconstituted with distilled water and autoclaved The sulfonamides (sulfadiazine sulfathiazole sulfisoxazole, sulfamethazine and sulfamerazine) and the sulfones (diazone promin and promizole) were tested Those insoluble in a 0 9 per cent solution of sodium chloride were diluted in either 1 N hydrochloric acid or sodium hydroxide Further dilutions were made in sterile normal saline and added to the medium to make the desired concentrations The Nocardia species were grown on Sabouraud s agar and triturated in saline for the inoculum The *A bovis* strains were grown in SR medium All cultures were incubated at 37°C Readings were made at five seven and ten days With only a few exceptions the end point was evident at five days

IN VIVO STUDIES

Strain 108 of *Nocardia asteroides* which was sensitive in vitro to 100 μgm of diasone and which was partially inhibited by 10 000 μgm of sodium sulfadiazine was used in animal studies Young white mice averaging 15 gm were inoculated intraperitoneally with 0 5 ml of the organism which was suspended 1 50 by volume in sterile 5 per cent hog gastric mucin Six hours later and then

daily for three days 0 5 ml of various concentrations of sodium sulfadiazine and diasone were given by intraperitoneal injection All mice surviving at one week were killed and examined for evidence of infection

RESULTS

IN VITRO STUDIES

Table 1 presents the results of this investigation The figures represent total inhibition of growth Partial inhibition was observed always at the next lower 10 fold dilution In some instances partial inhibition was noted at a 100-fold dilution The variation in susceptibility of each strain to the different drugs is clearly evident

The sulfones diasone and promizole are effective in controlling the growth of most of the strains of Nocardia tested Two strains of *A bovis* which appear insensitive to the sulfonamides were inhibited by these compounds

Sulfamerazine appears to be the most effective of the sulfonamide drugs in inhibiting the growth of *N asteroides* in vitro It produces complete inhibition at dilutions usually 10- and occasionally 100 fold less than other sulfonamides In general a strain which is sensitive to one drug at a low concentration is also comparatively sensitive to the other sulfonamides Other strains were by comparison sensitive only to high concentrations of the drugs

Strains 104 and 107 of *N madurae* are from the same patient strain 107 being isolated after seven months of intermittent sulfonamide therapy There has been no apparent change in sensitivity

IN VIVO STUDIES

The results of this study are presented in Table 2 The strain used produced only 80 per cent mortality in the control mice Those control animals surviving appeared ill and when sacrificed showed numerous lesions Examination of all infected control and test animals succumbing to the infection revealed many small nodules scattered throughout the organs of the peritoneal cavity There were adhesions between the diaphragm and liver and between the stomach spleen and peritoneal wall

TABLE 1

SENSITIVITY OF VARIOUS PATHOGENIC ACTINOMYCETES TO SULFONAMIDES AND SULFONES (IN MICROGRAMS PER MILLILITER OF MEDIA)

	Sulfadiazine	Sulfathiazole	Sulfisoxazole	Sulfamethazine	Sulfamerazine	Diasone	Promizole	Promin
Nocardia asteroides								
13	10 000	1000	1000	1000	1000	10	10 000	1000
33	1000	>10 000	>10 000	1000	1000	100	1	10
50	10 000	1000	>10 000	>10 000	>10 000	10	1000	10 000
92	10 000	1000	1000	10 000	100	100	10	1000
106	1000	100	10 000	1000	10 000	10	1000	1000
108	>10 000	10 000	>10 000	>10 000	10 000	100	1000	>10 000
111	10 000	100	10 000	1000	1000	100	1000	10 000
112	10 000	1000	10 000	1000	100	1000	10	1000
113	1000	1000	1000	1000	10	100	10	10 000
114	>10 000	100	1000	1000	1000	10 000	10	1000
115	>10 000	1000	>10 000	1000	10	10 000	1	10 000
116	>10 000	1000	10 000	1000	>10 000	10 000	10	1000
117	1000	1000	1000	100	100	100	100	1000
118	10 000	1000	10 000	10 000	100	>10 000	10	1000
119	100	100	10	100	1	100	1	1000
120	>10 000	1000	10 000	1000	10 000	>10 000	100	1000
126	1000	1000	10 000	>10 000	100	1000	>10 000	1000
127	>10 000	100	1000	100	100	10	10	1000
128	10 000	1000	1000	100	10	100	100	1000
129	1000	1000	1000	100	10	100	10	1000
130	100	10	100	1000	10	1000	10	1000
131	>10 000	1000	1000	1000	>10 000	10 000	100	10 000
132	10 000	1000	1000	1000	1000	1000	1000	10 000
Nocardia brasiliensis								
12	1000	>10 000	>10 000	100	10 000	10	1	10
123	1000	10	10 000	10	1	10	10	1000
124	1000	100	1000	1000	1000	1000	1000	1000
125	>10 000	100	1000	1	10	100	100	10 000
Nocardia madurae								
30	1000	100	1000	1000	100	1	10	1000
31	100	1000	1000	1000	>10 000	1000	100	1000
104	10	10	10	100	100	10	10	1000
107	10	10	10	100	100	10	10	1000
Nocardia pelletieri								
11	100	100	100	1000	1000	10	100	1000
136	1000	100	10 000	100	100	100	10	100
138	10 000	1000	100	1000	1000	100	>10 000	100
Actinomyces bovis								
1427	10 000	10 000	>10 000	>10 000	>10 000	>10 000	100	10 000
1647	>10 000	>10 000	>10 000	>10 000	>10 000	100	-	>100
2029	>10 000	>10 000	10,000	>10 000	>10 000	100	-	>100

The mice on diasone which survived the test period appeared healthy At autopsy however small nodules were found in all mice The mice receiving sodium sulfadiazine and surviving the test period presented more extensive lesions

Diasone in *10- and 50 mg doses appeared to be the most effective* of the drugs tested in vivo However larger concentrations of this drug apparently enhanced the mortality rate when introduced into

TABLE 2

EFFECT OF DIASONE AND SODIUM SULFADIAZINE ON SURVIVAL RATE OF MICE INFECTED WITH *NOCARDIA ASTEROIDES*

	Daily Dosage (mg /kg)	Number of Mice Injected	Deaths after day 1	2	3	4	5	6	Per Cent Survival
Control		10		8					20
Diasone	10	14		2	1	2			64
	50	14		2	2	3	1		43
	100	14	1	8	4			1	0
Drug control	100	5							100
Sodium sulfadiazine	10	14	2	8				1	21
	50	14	1	4	1	3			36
	100	14	1	1	2	1			64
Drug control	100	5							100

the inoculated animal Sodium sulfadiazine in 100-mg doses gave a large degree of protection At lower dosages a somewhat beneficial effect was also seen

DISCUSSION

Although strain variation in the susceptibility of pathogenic actinomycetes to sulfonamides and sulfones has been observed by Strauss et al [1] and by González Ochoa et al [2,3] sensitivity testing has rarely been used for the choice of drugs in treatment The relatively slow growth of these organisms and in the case of *A bovis* the necessity for anaerobiosis have further complicated these tests Moreover most media on which these organisms are routinely grown contain substances antagonistic to the action of these drugs However since Nocardia infections respond to sulfonamides and sulfones more readily than to any other form of medication procedures for determining which drug is most effective in vitro should be useful in the selection of the most desirable compound for treatment

Diasone and promizole appear to be the most effective of the test

TABLE 1

SENSITIVITY OF VARIOUS PATHOGENIC ACTINOMYCETES TO SULFONAMIDES AND SULFONES (IN MICROGRAMS PER MILLILITER OF MEDIA)

	Sulfadiazine	Sulfathiazole	Sulfisoxazole	Sulfamethazine	Sulfamerazine	Diasone	Promizole	Promin
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106	1000	100	10 000	1000	10 000	10	1000	1000
108	>10 000	>10 000	>10 000	>10 000	>10 000	100	1000	>10 000
111	10 000	100	10 000	1000	1000	100	1000	10 000
112	10 000	1000	10 000	1000	100	1000	10	1000
113	1000	1000	1000	1000	10	100	10	10 000
114	>10 000	100	1000	1000	1000	>10 000	10	1000
115	>10 000	1000	>10 000	1000	10	10 000	1	10 000
116	>10 000	1000	10 000	1000	>10 000	>10 000	10	1000
117	1000	1000	1000	100	100	100	100	1000
118	10 000	1000	10 000	10 000	100	>10 000	10	1000
119	100	100	10	100	1	100	1	1000
120	>10 000	1000	10 000	1000	10 000	>10 000	100	1000
126	1000	1000	10 000	>10 000	100	1000	>10 000	1000
127	>10 000	100	1000	100	100	10	10	1000
128	10 000	1000	1000	100	10	100	100	1000
129	1000	1000	1000	100	10	100	10	1000
130	100	10	100	1000	10	1000	10	1000
131	>10 000	1000	1000	1000	>10 000	10 000	100	10 000
132	>10 000	1000	1000	1000	1000	1000	1000	10 000
Nocardia brasiliensis								
12	1000	>10 000	>10 000	100	10 000	10	1	10
123	1000	10	10 000	10	1	10	10	1000
124	1000	100	1000	1000	1000	1000	1000	1000
125	>10 000	100	1000	1	10	100	100	10 000
Nocardia madurae								
30	1000	100	1000	1000	100	1	10	1000
31	100	1000	1000	1000	>10 000	1000	100	1000
104	10	10	10	100	100	10	10	1000
107	10	10	10	100	100	10	10	1000
Nocardia pelletieri								
11	100	100	100	1000	1000	10	100	1000
136	1000	100	10 000	100	100	100	10	100
138	10 000	1000	100	1000	1000	100	>10 000	100
Actinomyces bovis								
1427	>10 000	>10 000	10 000	>10 000	>10 000	>10 000	100	10 000
1647	>10 000	>10 000	>10 000	>10 000	>10 000	100	–	>100
[illegible]	>10 000	>10 000	>10 000	>10 000	>10 000	>100	–	>100

The mice on diasone which survived the test period appeared healthy At autopsy however small nodules were found in all mice The mice receiving sodium sulfadiazine and surviving the test period presented more extensive lesions

Diasone in 10- and 50 mg doses appeared to be the most effective of the drugs tested in vivo However larger concentrations of this drug apparently enhanced the mortality rate when introduced into

important as those carried out on bacteria in the choice of thera peutic agents Both sulfonamide and sulfone compounds should be tested along with antibiotics and possibly drugs found useful in the treatment of tuberculosis

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drugs in inhibiting the growth of pathogenic actinomycetes in vitro It is believed that sulfones inhibit bacteria because they break down in vivo into diamino diphenyl sulfone (DDS) This compound presumably has a mechanism of action similar to that of the sulfonamides González Ochoa et al [3] have shown this compound to inhibit the growth of Nocardia in vitro and have cured a number of infections due to *N brasiliensis* Garcia [4] has reported success in treating a case of nocardiosis with diasone

Studies in mice showed that diasone was of benefit only when used in low concentrations The interpretation of these results is difficult Actinomycotic diseases in man are not comparable to those in animals where infection can be induced only by drastic means (large volume of inoculum in gastric mucin) Recent clinical studies indicate that man can tolerate a fairly large dosage of the sulfones before symptoms of toxicity appear

Sulfadiazine has been used with success in treating many cases of Nocardia infection Although this study showed that this drug was relatively ineffective in vitro against most strains of *N asteroides*, sulfadiazine in a fairly large dose significantly lowered the death rate in mice infected with one of the nonsensitive strains Apparently fungistatic action at concentrations below that required for complete inhibition of growth is all that is needed to enable the host s natural defense mechanism to overcome the infection

Prior to the use of antibiotics for treatment of infections due to *A bovis* the sulfonamides had been used with varying success Many cases where sulfonamides alone were employed have met with failure However where sulfonamide therapy was supported by surgical intervention x ray therapy and the administration of iodides cure has more often been recorded Arnold [5] has reported a successful cure of cervical facial actinomycosis with diasone after having first observed similar treated cases by Latapi

Cutting and Gebhardt [6] reported complete inhibition of *A bovis* with 500 μgm of sulfathiazole and sulfadiazine per milliliter of media whereas Keeney Ajello and Lankford [7] were able to achieve inhibition only in concentrations of 5000 μgm per milliliter The three strains of *A bovis* in this study were completely insensitive to the sulfonamides but two were sensitive to two sulfone compounds The strain variation in reaction to various drugs suggests that sensitivity tests for pathogenic actinomycetes are as

of chlorquinaldol (Sterosan) * as an intestinal antiseptic in pre surgical preparation of the large bowel

METHODS

The effect of chlorquinaldol was studied in 16 adult hospitalized patients who were normal from the gastrointestinal point of view and who had received no antibiotics or sulfonamides for at least four weeks prior to this study The first group of 4 patients was given 600 mg of chlorquinaldol in three divided doses for one day This dosage was chosen because it had previously been used thera peutically [6] To determine whether increased dosage would produce better or more consistent results than those obtained with 600 mg a second group of 8 patients received this medication at the level of 1 6 gm of chlorquinaldol in four divided doses for one day

Since chlorquinaldol was found to exhibit greater activity against the gram positive flora in the stools than against the gram negative organisms it was deemed advisable to combine it with a drug inhibiting the gram negative organisms As neomycin † has demonstrated these necessary properties [7, 8] this antibiotic was used in combination with chlorquinaldol One gram of neomycin four times a day in addition to 200 mg of chlorquinaldol four times a day was administered to 4 patients the total medication thus be ing 4 gm of neomycin and 800 mg of chlorquinaldol per day

CULTURE METHODS

The general culture methods employed in this study have been reported previously [1] Control stools were secured from all patients before medication was initiated Stool specimens were collected in clean containers and immediately sent to the laboratory where if storage was necessary before processing they were placed in a deep freeze unit at −20 C

One gram of wet stool was weighed into a sterile flask and emul sified with a known volume of physiologic saline solution The ho

Ge gy Pharmaceuticals kindly furnished the chlorquinaldol employed in this investigation under the trade name of Sterosan Outside the United States this intestinal ant septic s marketed under the Geigy trademark Siosteran

† Neomycin under the trade name of Mycifradin Sulfate was supplied through the courtesy of the Upjohn Company

BORIS A SHIDLOVSKY, MILTON MARMELL, ROBERT TURELL, and AARON PRIGOT

The Effect of Chlorquinaldol Alone and in Combination with Neomycin on the Intestinal Microbial Flora of Man Preliminary Report*

As new chemotherapeutic agents are introduced they must be subjected to therapeutic evaluation before being accepted for routine use Although good therapeutic agents for presurgical preparation of the bowel are available [1] there still exists a need for a drug or combination of drugs which will provide the maximum reduction of intestinal flora with minimal or no side reactions

Recently chlorquinaldol (5 7 dichloro 8 hydroxy quinaldine) was reported not only as being active against gram positive bacteria [2] but also as possessing fungistatic properties [3] Due to these properties chlorquinaldol appeared to be of value either alone or in combination with other drugs for intestinal antisepsis

Chlorquinaldol is a yellowish synthetic crystalline powder which is not readily soluble in water However it is soluble in alcohol ethyl acetate benzine petroleum ether and propylene glycol and other carbowaxes It is recovered almost completely in the feces [4] Brun et al [3] using filter paper impregnated with chlorquinaldol found it to be fungistatic whereas Wegmann and his associates [2] and Canaan [5] presented data indicating that this drug is principally active against gram positive bacteria

The present study was undertaken to evaluate the potentialities

* This investigation was supported in part by grants from Geigy Pharmaceuticals Division of Geigy Company Inc. New York City and the Upjohn Company Kalamazoo Michigan

by approximately 60 per cent in the remaining 2 patients Results on the yeastlike population were similarly variable ranging from insignificant reduction in 1 patient to over 85 per cent in the re maining 3 patients

When the dosage of chlorquinaldol was increased to 400 mg given four times in one day for a total of 1 6 gm (Fig 2) the re-

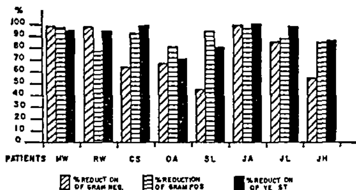


FIGURE 2

duction in the gram positive flora which included enterococci was approximately 80 to 90 per cent in 7 of the 8 patients The effect on the gram negative flora (*Escherichia coli* Proteus *A faecalis*, and Paracolon bacillus) was variable and ranged from 45 to 95 per cent The yeastlike population was reduced from 72 to 95 per cent in all these patients

The smaller dosage of 800 mg of chlorquinaldol given in four divided doses was combined with 1 gm of neomycin given four times a day for only one day (Fig 3) This combination produced over 90 per cent reduction in both the gram positive and the gram negative flora This reduction occurred within twenty four hours following the administration of the medication These results were consistent in all of the 4 patients The yeastlike organisms were re duced between 75 and 99 per cent in all cases

Chlorquinaldol displayed no toxic or side effects in any of the pa tients treated

mogenous suspension was diluted with sterile physiologic saline to give dilutions ranging from 10⁻ to 10^{-10} One milliliter aliquots of appropriate dilutions were then used to make pour plates with Endo agar blood agar azide agar, and Littman s ox gall agar One milliliter of an appropriate dilution was placed in thioglycollate broth The thioglycollate growth was used to isolate and identify the various micro organisms present in the given stool specimens These isolates were later used to determine their sensitivities to chlorquinaldol and neomycin alone and in combination

Counts of coliforms lactose nonfermenting bacilli including Proteus *A faecalis,* and Paracolobacterium gram positive cocci and yeasts were thus obtained

RESULTS

The results of medication in the first group of patients who re ceived 600 mg of chlorquinaldol in three divided doses for one day are summarized in Figure 1 This dosage reduced the gram positive

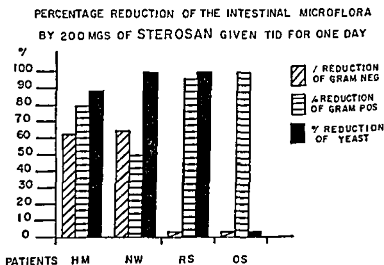


FIGURE 1

flora in 2 of the 4 patients by over 95 per cent In the third patient the reduction in these micro-organisms was 80 per cent and in the fourth patient it was only 50 per cent The effect on the gram negative flora was insignificant in 2 patients but they were reduced

concentrations of either of the drugs alone This effect is being investigated further

The effect of this combination on the yeastlike organisms and fungi suggests its possible usefulness in the treatment of disorders of the alimentary tract caused by these organisms

The problem of the emergence of resistant strains is in the process of further evaluation although we have not encountered any to date

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PERCENTAGE REDUCTION OF THE INTESTINAL MICROFLORA
BY I GRAM OF NEOMYCIN WITH 200 MGS OF STEROSAN
GIVEN QID FOR ONE DAY

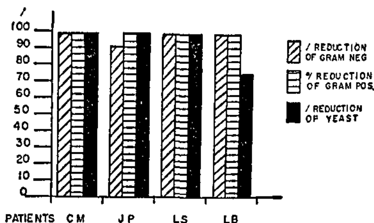


FIGURE 3

DISCUSSION

It is obvious that the intestinal contents of man are not sterilized by chlorquinaldol alone Even in the large dose of 1 6 gm of chlor quinaldol enterococci were not consistently reduced in all of our patients The effect on the gram negative organisms was relatively unpredictable However when this drug was combined with neomycin over 90 per cent of both the gram positive and gram nega tive organisms were reduced consistently

It is of interest that the chlorquinaldol neomycin combination not only enhances the reduction of the gram negative organisms but also potentiates the reduction of the gram positive intestinal bacteria This potentiation produces results which exceed those ob tained using the large dose of 1 6 gm of chlorquinaldol for one day This marked reduction is also observable in the yeastlike population in stool samples This result suggests that the mechan ism of this potentiation is synergistic

Similar results were demonstrated in in vitro studies The enter ococci isolated from stools were inhibited by smaller concentra tions of the chlorquinaldol neomycin combination than by larger

concentrations of either of the drugs alone This effect is being in vestigated further

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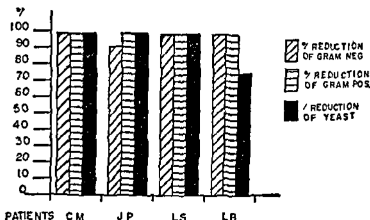


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Similar results were demonstrated in in vitro studies The enterococci isolated from stools were inhibited by smaller concentrations of the chlorquinaldol neomycin combination than by larger

kept in an ice bath during the period of inoculation as suggested earlier by Rowley As a further check on the possible loss of via bility 50 per cent of the untreated control mice were infected at the beginning and 50 per cent at the end of a series of inocu lations

The crude drug (lots 5 and 12) was supplied by NEPERA Nepera Park New York and the Antibiotics Division Food and Drug Administration Washington D C Insoluble in water it was *administered intraperitoneally as an aqueous suspension which was* prepared just prior to injection Individual doses were contained in 0 5 ml and ranged from 0 5 to 2 0 mg

RESULTS

As shown in Table 1 77 5 per cent of 40 mice infected with *S schenckii* and treated with a total dosage of 10 mg (group 2) and 62 5 per cent of those receiving 8 5 mg (group 3) of lot 5 sur vived through the fourteenth day as compared to 20 per cent in infected untreated controls (group 1) Although death rates in creased in both treated groups thereafter the total mortalities were still reduced in these groups on the twenty first day A more ex tended period of protection was observed in group 5 where 70 per cent of the mice treated with the 10-mg total dosage survived through the twenty-eighth day and 50 per cent through the forti eth A mortality of 65 per cent on the fourteenth day in the control

TABLE 1

THERAPEUTIC EFFECT OF ANTIBIOTIC 1968 (LOT 5) IN EXPERIMENTAL SPOROTRICHOSIS

Group	*No in Group*	*Daily Dosage (mg)*	*Total Dosage (mg)*	*Percentage Fatality on Day*				
				7	*14*	*21*	*28*	*40*
1	40	—	—	0	80	97 5	97 5†	
2	40	1 0	10	0	22 5	65 0	80 0†	
3	40	0 5	8 5	5	37 5	75 0	85 0†	
4	20	—	—	0	65	100		
5	20	1 0	10	0	0	15	30	50

Drug administered at time of infection and IX daily thereafter
† Experiment terminated on this date

CHARLOTTE C CAMPBELL

Preliminary Results with a New Antibiotic, 1968 (NEPERA), in Mice with Experimental Histoplasmosis, Sporotrichosis, and Candidiasis

Preliminary studies with an antibiotic derived from *Streptomyces* sp 1968 (NEPERA) reveal that it possesses therapeutic activity in mice experimentally infected with *Histoplasma capsulatum Sporotrichum schenckii* or *Candida albicans*

The experiments were carried out in groups of male Swiss albino mice (O Grady strain) weighing 18 to 20 gm which were treated once daily for ten days with varying concentrations of the drug beginning at the time of infection Evaluation of therapeutic activity was based on the prolongation of survival time of treated as compared with untreated animals Unless otherwise noted experiments were terminated on the twenty eighth day after infection

Inoculating suspensions were prepared in cold physiologic saline solution from forty eight hour yeast phase cultures of *H capsulatum* (G–8) *S schenckii* (F–20) and *C albicans* (E–70) grown on brain heart infusion agar at 37°C The procedure consisted of resuspending washed cells to a 20 ml volume followed by centrifugalization at 500 rpm for five minutes to remove the larger aggregates and by further dilution of this supernate to a desired light transmission on the Coleman spectrophotometer In these studies suspensions of *H capsulatum S schenckii*, and *C albicans* which transmitted 50 70 and 90 per cent light at 500 mμ respectively were employed The inoculum for a mouse was 0 2 ml and was administered by the intravenous route As determined by preliminary experiment this dose resulted in a mortality of 70 to 90 per cent by the fourteenth day The inoculating suspensions were

the twenty-eighth day in contrast to a 90 per cent mortality in untreated controls by the fourteenth day (group 6)

The data in Table 3 reveal that the therapeutically active substance at *Streptomyces* sp 1968 is extractable by methanol but not by water The survival time of mice infected with *H capsulatum* was prolonged in groups 11 12 and 13 which were treated with total dosages of 20 mg of the first second and third methanol extractions respectively Equivalent concentrations of five successive aqueous extractions (groups 15 and 16) made prior to the methanol extractions were ineffective The fourth fifth and sixth methanol fractions (group 14) also failed to suppress mortality

TABLE 4

THERAPEUTIC EFFECT OF ANTIBIOTIC 1968 (LOT 12) FRACTIONS G AND H IN EXPERIMENTAL CANDIDIASIS

Group	No in Group	Daily Dosage (mg)	Total Dosage (mg)	Percentage Fatality on Day			
				7	14	21	28
17	20	—	—	20	70	90	90
18	20	1 5	15	0	5	20	20
19	20	1 0	10	0	0	25	35

1 mg administered at time of infection and 1X daily for nine days

Small aliquots of fractions G and H remained after completion of the preceding study and were combined for further investigation in experimental candidiasis These results are shown in Table 4 Of mice treated with total dosages of 10 and 15 mg (groups 18 and 19) 65 and 80 per cent respectively survived the twenty eighth day observation period By contrast there was a 70 per cent mortality in the untreated controls on the fourteenth day

The prolongation of survival time in mice infected with lethal doses of *H capsulatum S schenckii* or *C albicans* effected by the administration of experimental antibiotic 1968 in adequate concentration suggests that this drug warrants further investigation as a potential therapeutic agent for these mycoses in man The drug is nontoxic for mice in the concentrations employed

Efforts to purify the drug as well as studies to determine possible relationship to Ascosin or Mycostatin are in progress

TABLE 2

THERAPEUTIC EFFECT OF ANTIBIOTIC 1968 (LOT 5) IN EXPERIMENTAL HISTOPLASMOSIS *

Group	*No in Group*	*Daily Dosage (mg)*	*Total Dosage (mg)*	*Percentage Fatality on Day*			
				7	*14*	*21*	*28*
6	20	—	—	0	90	95	95
7	20	2 0	20	5	15	35	35
8	20	1 0	10	10	35	70	70
9	20	0 5	5	10	65	85	95

* *Drug administered at the time of infection and IX daily thereafter*

animals for this experiment (group 4) suggests that the infections in groups 4 and 5 were less severe than those in groups 1 2 and 3

The therapeutic activity of antibiotic 1968 (lot 5) in mice infected with a rapidly lethal dosage of *H capsulatum* is illustrated in Table 2 Survival time was significantly prolonged beyond the fourteenth day in the animals receiving total dosages of 10 (group 8) and 20 mg (group 7) less so in those treated with a total dosage of 5 mg (group 9) The protective activity was most marked in group 7 which received the maximum total dosage of 20 mg employed in these studies In this group 65 per cent survived through

TABLE 3

THERAPEUTIC ACTIVITY OF METHANOL AND WATER EXTRACTABLE FRACTIONS OF STREPTOMYCES SP 1968 (LOT 12) IN MICE INFECTED WITH *HISTOPLASMA CAPSULATUM* *

Group	*Fraction No*	*Type of Extraction*	*Percentage Fatality on Day*			
			7	*14*	*21*	*28*
10	—	—	0	70	85	90
11	G*	Methanol 1	0	20	65	75
12	H*	Methanol 2	5	5	30	35
13	M*	Methanol 3	5	35	55	60
14	N*	Methanol 4, 5 and 6 (combined)	5	75	95	100
15	A*	Water 1	0	90	100	100
16	B*	Water 1–5 (combined)	0	80	90	95

* 1 mg administered at time of infection and once daily for nine days

the twenty-eighth day in contrast to a 90 per cent mortality in un treated controls by the fourteenth day (group 6)

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Efforts to purify the drug as well as studies to determine possible relationship to Ascosin or Mycostatin are in progress

Nystatin and Actidione Two Antifungal Agents Produced by *Streptomyces noursei*

Investigations have been concerned with the two antifungal agents that are produced by *Streptomyces noursei*[1] In the early work with this actinomycete nystatin was recognized even in crude form as a new antibiotic because of its broad antifungal spectrum More recently the second antibiotic has been identified by chemical and physical means as actidione[2,3]

The simultaneous production of these two antifungal agents by *S noursei* is of interest Subsequent to the discovery of nystatin other antibiotics related to it have been reported in particular antimycoin[4] which is very similar but which is produced by *S aureus* Actidione had been isolated previously from *S griseus* broth cultures that produce streptomycin also

Under conditions for static growth the two antibiotics are produced simultaneously They can be separated crudely by straining out the mycelium Nystatin the less soluble of the two is found here while actidione a water soluble antibiotic is present in the culture liquors The yield of actidione averages ten times that of nystatin Both antibiotics have been obtained in crystalline form

The great difference in solubilities of the two products makes possible a ready separation nystatin is almost insoluble in water slightly soluble in lower aliphatic alcohols and glycols One of the most satisfactory neutral solvents is 70 per cent ethanol Actidione on the other hand is readily soluble in water and many organic solvents

Nystatin is most stable as a dry powder In this state it has been stored under refrigeration for four years without appreciable loss of activity In solution nystatin is unstable to heat acid and alkali Alcoholic solutions begin to deteriorate within one week under

ordinary refrigeration Aqueous suspensions may be stored in the frozen state at −25°C however for at least two months without deterioration

Actidione is more stable to heat an aqueous solution at 100 C began to lose activity only after 1 5 hours Acid and alkaline solutions stored at 4° to 8°C maintained their activity for four weeks

TABLE 1

ANTIFUNGAL SPECTRA OF NYSTATIN AND ACTIDIONE

Fungi	Least Amount Inhibiting Growth (μg/ml)	
	Nystatin	Actidione
Candida albicans	3 13	> 400
Candida guilliermondi	3 13	> 400
Monosporium apiospermum	200	> 400
Blastomyces dermatitidis (yeast phase)	1 56	> 400
Histoplasma capsulatum (yeast phase)	1 56	> 400
Geotrichum lactis	6 25	> 400
Trichophyton mentagrophytes	6 25	> 400
Cryptococcus neoformans	1 56	3 13
Aspergillus fumigatus	6 25	400
Penicillium sp	13	100
Saccharomyces cerevisiae	3 13	< 0 78
Candida krusei	6 25	< 0 78
Saccharomyces pastorianus	3 13	0 39
Rhodotorula mucilaginosa	1 56	0 39

Chemically the two antibiotics are unrelated Both are nitrogen containing compounds The ultraviolet spectrum of nystatin shows three marked bands at 291 305 and 319 mμ indicative of a polyene the spectrum of actidione shows weak absorption at 287 mμ

The antifungal spectra of the two antibiotics as determined by the agar dilution method differ markedly (See Table 1) Of the many fungal pathogens sensitive to nystatin only *Cryptococcus neoformans* is sensitive to actidione The nonpathogenic yeasts are somewhat more sensitive to actidione than to nystatin Effective doses of nystatin range from 1 56 to 13 μg/ml those of actidione from 0 39 to 3 13 μg/ml

Both agents are strongly fungistatic and fungicidal In a concen

tration of 12 5 μg /ml the fungicidal effect of nystatin is greater than that of actidione

The toxic effects of the two antibiotics are quite different in mice For nystatin the acute LD_{50} is 20–26 mg /kg by the intra peritoneal route and greater than 150 mg /kg by the subcutaneous route Repeated subcutaneous injections are accompanied by ne crosis but no gross cumulative toxic effects were noted following injections of a total of 1250 mg /kg during a period of eight days

For actidione the acute LD_{50} is 138 mg /kg by the intraperi toneal route and 110 mg /kg by the subcutaneous route There is evidence for cumulative toxic effect upon repeated intraperitoneal injections of sublethal doses

Nystatin is proving to be of value as an antifungal agent both in vitro and in vivo Because of its very narrow spectrum actidione is of less value

At the Division of Laboratories and Research * nystatin is used routinely in tissue culture techniques A concentration of 10 μg /ml is employed for the preparation of throat and fecal in ocula for the isolation of viruses This concentration has not been toxic for HeLa monkey kidney or normal human epithelial cells HeLa cells were maintained continuously in the presence of ny statin at this concentration for five months without damage For the examination of sewage and polluted waters for enteric viruses by means of isolation on HeLa cell culture 20 μg /ml is added to the maintenance medium The antibiotic is also useful in the iso lation of bacteria from pathologic specimens contaminated with yeasts and molds

There are many reports of protection afforded by nystatin to mice infected with several of the pathogenic fungi Ninety per cent of infected mice were protected against cryptococcosis and 80 per cent against histoplasmosis whereas all untreated animals died[5] Newcomer and others[6] reported that 80 per cent of mice infected with *Coccidioides immitis* were protected when none of the un treated mice survived Campbell and others[7] reported 27 5 per cent protection of mice infected with *Sporotrichum schenckii*, while none of the controls survived Sternberg and others reported 92 per cent protection of mice infected with *Candida albicans* when only 27 5 per cent of the untreated mice survived When the

* New York State Department of Health.

moniliasis was induced by injection of *C albicans* mixed with chlortetracycline[9] or with oxytetracycline[10] and the mice were treated with nystatin 53 per cent and 73 per cent respectively were protected whereas there were no survivals among the un treated mice

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tration of 12.5 µg/ml the fungicidal effect of nystatin is greater than that of actidione.

The toxic effects of the two antibiotics are quite different in mice. For nystatin the acute LD_{50} is 20–26 mg/kg by the intraperitoneal route and greater than 150 mg/kg by the subcutaneous route. Repeated subcutaneous injections are accompanied by necrosis but no gross cumulative toxic effects were noted following injections of a total of 1250 mg/kg during a period of eight days.

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hols is increased by the presence of 10 to 20 per cent of water The compound has considerable solubility in pyridine dimethylformamide glacial acetic acid and 0 05 N methanolic hydrochloric acid or sodium hydroxide although in these solvents it suffers rather rapid inactivation

The specific rotation of nystatin is −8 in glacial acetic acid and +21° in pyridine The analytic data are in good agreement for an empirical formula of $C_{46}H_{77}NO_{19}$ and show the absence of any

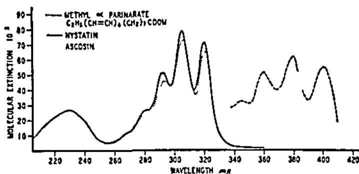


FIGURE 1

halogen or sulfur as well as any methoxyl ethoxyl or N methyl groups Analysis has indicated the presence of four C-methyl groups

Figure 1 shows the ultraviolet absorption spectrum of nystatin together with methyl α parinarate a compound containing four conjugated double bonds Both have absorption maxima at 292 mμ 305 mμ and 320 mμ and the close resemblance of the two spectra leaves no doubt that they both contain the same chromophore The very slight maximum at 230 mμ in the case of methyl α parinarate is typical of these conjugated systems and is called the cis peak The enhanced absorption of the nystatin in this same region is best ascribed to the absorption of a dienic system superimposed on the cis peak As will be seen later there is further evidence that nystatin contains two double bonds in addition to the four involved in the conjugated tetraene system As Oroshnik et al [3] have pointed out this type of ultraviolet absorption spectrum with its characteristic fine structure is typical of a whole class of polyenic anti

J D DUTCHER, D R WALTERS,
and O P WINTERSTEINER

Studies of the Chemical Properties and Structure of Nystatin (Mycostatin)

Nystatin the antifungal antibiotic discovered by Drs Hazen and Brown [1] in 1951 which ordinarily is obtained as an amorphous or partially crystalline product has been obtained in pure form as very fine needles This purified material has the physical properties listed in Table 1 It is insoluble in nonpolar solvents such as hexane and chloroform and only slightly soluble in water, methanol ethanol and butanol The solubility of nystatin in the alco-

TABLE 1

PHYSICAL PROPERTIES OF CRYSTALLINE NYSTATIN

A Solubility

1 Insoluble in acetone chloroform, or ether
2 Slightly soluble in water methanol, ethanol or butanol Solubility increased in water alcohol mixtures
3 Soluble in pyridine, glacial acetic acid, dimethylformamide, 0 05 N methanolic HCl or NaOH The last three solvents however rapidly inactivate Nystatin

B Melting Point

Gradual decomposition above 165°C No definite melting

C Specific Rotation

$[\alpha]_D^{25}$ −8° (glacial acetic) +21° (pyridine)

D Analysis

Calcd for $C_{46}H_{77}NO_{19}$ (M W = 948)	C, 58 27 H, 8 18 N 1 48
Found	C, 58 42 H, 8 18 N, 1 66
	C 58 58 H 8 28 N 1 62

N E = 955, 956 C methyl 5 86 halogen sulfur, methoxy acetyl N methyl, negative

of a carboxylate group The ionic character of this latter group is indicative of the fact that in the crystalline form nystatin must exist as a zwitter ion As chemical evidence for the presence of a carbonyl group it has been demonstrated that the compound re acts stoichiometrically with one equivalent of methoxide ion in methanol and reacts with diazomethane to form a mono methyl ester The nitrogen exhibits its basic nature on titration with perchloric acid in glacial acetic acid Also the basicity is lost upon acetylation The fact that ammonia is evolved when nystatin is

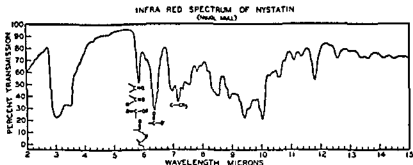


FIGURE 2

heated with strong alkali suggests that the nitrogen may be present in the molecule as a primary amino group

Since the hydrogenation experiments indicate the absence of any reducible carbonyl function such as an aldehyde or ketone group the infrared band at 5 87 μ must be due to either a lactone or ester group Of these two possibilities the lactone seems most probable since it has been observed that in the presence of excess base both nystatin and its hydrogenated derivative rapidly consume two moles of base with concomitant loss of the carbonyl band in the infrared This consumption of two moles can be accounted for by reaction of the first mole with the carbonyl group and the uptake of an additional mole of base by hydrolysis of the lactone group The rate of this hydrolysis is much greater than would be exacted for an ordinary ester

Table 2 lists some additional chemical properties of nystatin and the dodecahydro compound The reaction between nystatin and a mixture of acetic anhydride and pyridine leads to a difficultly sepa rable mixture of acetylated compounds from which no clean prod

fungal agents which have been discovered in recent years mostly as products of various Streptomyces fermentations The locations of the maxima are useful in determining the exact nature of the chromophore The ultraviolet spectrum of Rimocidin an antibiotic isolated by Davisson et al [3] is practically identical with that of nystatin and even the chemical composition of the two is quite similar However they do differ in their general biologic and chemical properties The spectrum of Ascosin included in Figure 1 also showed the triad of maxima but at wave lengths which indicate the chromophore to be a conjugated heptaene In its general chemical characteristics it also appears quite similar to nystatin but of course the two can be differentiated by means of their ultraviolet spectra Candidin the antifungal agent discovered by Waksman and his co workers [4] has its maxima at the same wave lengths as Ascosin Since the latter two antibiotics have not yet been obtained pure their specific extinctions are not known and therefore the curve of Ascosin shown here is intended to indicate merely the position of the peaks The extinctions of the pure antibiotic would almost certainly be greater than depicted here

The unsaturated nature of nystatin has been confirmed by catalytic hydrogenation In the presence of either Adams platinum catalyst or 5 per cent palladium on carbon nystatin takes up 6 moles of hydrogen per mole of compound 2 moles in excess of that required to saturate the tetraene system Since 5 per cent palladium on carbon is capable of effecting only the hydrogenation of carbon carbon double bonds and since the ultraviolet absorption is abnormally intense in the region of dienic absorption this finding confirms the presence of a conjugated diene system in the nystatin molecule The difference in extinction at 231 mμ between nystatin and a normal tetraene system is equal to the intensity of absorption one would expect for a conjugated diene system The dodecahydronystatin has only end absorption in the ultraviolet and has no antifungal activity It is readily crystallized possesses greater chemical stability than nystatin itself and therefore is more suitable for certain structural investigations

The infrared absorption spectrum of nystatin is shown in Figure 2 Besides a broad intense hydroxyl band at 3 0 μ the strong bands at 5 87 μ and 6 37 μ are very significant The lower band is characteristic of a carbonyl function and the higher one is typical

and various peroxides and epoxides show a positive response When pure nystatin reduces neither Fehling s nor Tollens reagent and does not form a 2 4-dinitrophenylhydrazone The latter tests are additional evidence against the presence of a reducible carbonyl group

Since there are nineteen oxygen atoms in nystatin with only four accounted for by the carboxyl and lactone groups it is obvious that the compound is heavily hydroxylated This immediately suggests the possibility of there being a carbohydrate moiety in the structure perhaps attached through a glycosidic linkage to the remainder of the molecule However both nystatin and the dodecahydro derivative give only a faint Molisch test and under hydrolytic conditions which normally cleave glycosidic linkages namely heating with methanolic hydrochloric acid nystatin yields no carbohydrate fragments Numerous attempts to cleave the molecule in this manner have all failed The negative Millon s phenol test indicates that none of the hydroxyls are phenolic in nature

When nystatin is reacted with base several different reactions may occur depending upon the concentration of the reagent In the presence of just one equivalent of sodium hydroxide the sodium salt of the carboxylic acid is formed This compound has a greater solubility in water than nystatin and still is biologically active but it is relatively unstable A second equivalent of base cleaves the lactone group and at higher alkali concentrations a base-catalyzed polymerization occurs and the biologic activity is rapidly destroyed This latter reaction does not occur with the hydrogenated compound

With acids nystatin forms the corresponding salts which are water soluble They also lose their antifungal activity if stored for relatively short periods of time

Before an audience whose primary interest in nystatin is its therapeutic application it seems appropriate to make a few additional comments about the solubility and stability of this substance The solubility properties given in Table 1 indicate that considerable difficulty might be encountered in the preparation of parenteral solutions having a significant concentration of nystatin However if instead of employing crystalline nystatin one uses lyophilized nystatin the situation is greatly improved The lyophilized material is much more soluble in neutral solvents than its crystalline

uct could be isolated However the hydrogenated derivative yielded under similar conditions a chloroform soluble product which could be purified by chromatography on alumina Analysis showed it to contain six acetyl groups one of which was on nitrogen since the product was neutral

With potassium periodate in neutral solution or lead tetraace

TABLE 2

CHEMICAL PROPERTIES OF NYSTATIN AND DODECAHYDRONYSTATIN

	Nystatin	*Dodecahydronystatin*
Acetylation with acetic anhydride and pyridine	Causes considerable decomposition	Hexa acetyl derivative
Periodate oxidation	Unspecific	Consumes 3 moles
Lead tetra acetate oxidation	Unspecific	Consumes 3 moles
Schiff aldehyde test	Positive (atypical)	Negative
Reaction with 2,4 Dinitro phenyl hydrazine	Negative	Negative
Fehling or Tollens test	Negative	Negative
Molisch carbohydrate test	*Faint*	*Faint*
Millon phenol test	Negative	Negative
Reaction with alkali	Alkaline salts	Alkaline salts
Reaction with acid	Acid salts	Acid salts

tate in acetic acid nystatin was oxidized in an unspecific manner and consumed up to 10 moles of either reagent Dodecahydronystatin under the same conditions consumed 3 moles rapidly and further oxidation proceeded only slowly When the reaction was carried out stoichiometrically with a 3 mole uptake 1 mole of formaldehyde was isolated from the reaction mixture as the dimedon derivative The main product of the reaction is under investigation at the present time

Nystatin gives a slow positive reaction with the Schiff test which we believe to be atypical The lack of specificity of this test has been demonstrated previously by the observation that vinyl amines

3 DAVISSON J W TANNER F W FINLAY A C JR and SOLOMONS I A Rimocidin a new antibiotic Antibiotics & Chemotherapy 1 289 1951

4 TABER W A VINING L C and WAKSMAN S A Candidin a new antifungal antibiotic produced by *Streptomyces viridoflavus* Antibiotics & Chemotherapy 4 455 1954

counterpart For example it is soluble in propylene glycol to the extent of 100 000 units per milliliter If still higher concentrations of nystatin are desired it is expedient to use stabilized suspensions of the lyophilized material

It is easy to understand that nystatin which contains diene and tetraene systems as well as a lactone group would exhibit some in stability toward extremes of pH and also to light and oxygen When neutral solutions of nystatin are allowed to stand at room temperature in ordinary sunlight as much as 30 per cent of the ac tivity may be lost in three days 40 per cent in a week and 50 per cent after two weeks Under refrigeration these solutions show much greater stability As would be expected nystatin is more sta ble in the solid state than in solution Crystalline nystatin is stable indefinitely at room temperature The lyophilized powder under goes some inactivation about 20 per cent during the first two weeks but after that the potency remains essentially constant for months Both light and oxygen are involved in the inactivation process but the two effects are independent of each other

Unfortunately our investigation has not yet led to the develop ment of a satisfactory and convenient chemical assay procedure The prospect of employing the ultraviolet absorption as an assay method had to be discarded when the lack of strict correlation be tween the intensity of absorption and biologic activity was demon strated Under various conditions samples of nystatin showed al most maximum absorption intensity after a considerable portion of the activity was lost

Our investigation into the chemical nature of nystatin will con tinue We feel that dealing as we are with one member of a group of polyenic antifungal agents the knowledge derived from nystatin might very well be of value in elucidating the structures of the other antibiotics of this type

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2 Oroshnik W Vining L C Mebane A D and Taber W A Polyene antibiotics Science 121 147 19ɔ5

pleted by shaking If the solution is then permitted to stand for several hours at room temperature sterilization is generally effected through the action of the alcohol

(2) For many uses a solution of 10 to 100 units per milliliter is required but the alcohol contained therein may have undesirable

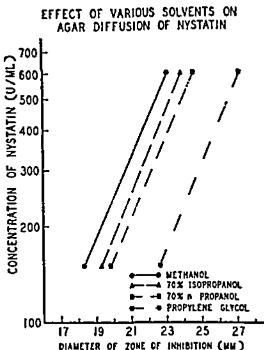


FIGURE 1

effects at the levels added In such cases a second procedure may be preferred Here 10 mg or more of nystatin is weighed into a sterile container and rubbed to form a smooth paste with 1 ml of 70 per cent alcohol The paste is permitted to stand for several hours at room temperature for sterilization and is then diluted to the desired volume with sterile distilled water The resulting suspension containing only about 1 per cent alcohol may be added to most biologic materials without fear of effects from the solvent Nystatin is soluble to the extent of at least 300 units per milliliter in most aqueous media

It is obvious that not all contaminating organisms are necessarily

R DONOVICK, F E PANSY, H A STOUT,
H STANDER, M J WEINSTEIN, and W GOLD

Some in Vitro Characteristics of Nystatin (Mycostatin)

PREPARATION OF SOLUTIONS OR SUSPENSIONS

Because of the limited solubility of nystatin in water certain problems arise in attempting to carry out in vitro studies with this antibiotic Unless these problems are circumvented or overcome the results of in vitro studies may be very misleading As an example varying the organic solvent employed may lead to different rates of diffusion of the antibiotic in agar This is exemplified in Figure 1 In these experiments crystalline nystatin was dissolved in four different solvents and diluted in each case with the same solvent to give various concentrations of antibiotic The rates of diffusion from paper disks placed on agar plates were then determined by measuring the zones of inhibition using *Candida albicans* as the test organism It can readily be seen that attempting to compare antibiotic activity of a given weight of pure nystatin in two different solvents may lead to anomalous results

The rate of solution of nystatin is markedly affected by the particle size of the dry powder and amorphous preparations dissolve more readily than do those consisting of relatively large crystals For preparing sterile solutions to be used in various in vitro procedures for example in tissue culture studies several procedures have proved useful

(1) One to 2 mg of nystatin are weighed into a sterile container and then rubbed to produce a smooth paste with a few drops of 70 per cent ethanol or 70 per cent isopropanol A solution containing 2000 to 4000 units per milliliter may then be made by bringing to a final volume of 1 ml with the same solvent Any undissolved granules are crushed with a glass rod and final solution is com

mg of yeast extract per milliliter were added various concentrations of the sodium salts of the fatty acids to be tested *Candida albicans* was then added to give an initial count of *ca* 500 cells per milliliter Nystatin in the form of an n propanol solution was added in two-fold dilutions the tests were incubated at 30°C for

TABLE 1

EFFECTS OF VARIOUS AGENTS ON NYSTATIN ACTIVITY IN VITRO *

Agent	Concentration mg/ml	MIC of Nystatin units/ml
none	-	4 0
caprylate	0 10	4 0
oleate	0 50	8 0
"	0.10	8.0
linoleate	0 50	12.0
"	0 25	12 0
"	0 10	8 0
ricinoleate	0 25	6 0
"	0 10	6 0
yeast extract	5 0	4 0
casamino acids	5 0	4 0
Tween 80	0 50	4 0

*Test organism - Candida albicans

eighteen hours and the MIC was determined Casamino acids yeast extract and Tween 80 were tested in a similar fashion

It is evident from the data shown that the unsaturated fatty acids tested lowered the activity of nystatin whereas the saturated compound caprylate had no effect Yeast extract casamino acids and Tween 80 were also inactive On the basis of studies carried out in another laboratory in the Squibb Institute there are indications that unsaturated fatty acids may contribute to the instability of nystatin (Table 2) but in short term tests this is apparently not

250 mg ZnSO 1 0 mg $MnCl_2$ 1 0 mg $FeCl_3$ 0.5 mg $CuSO_4 \cdot 5H_2O$ 0 1 mg KI 100 γ 0 1 mg *l* asparagine 5 g hydrolyzed casein 200 mg inositol 5 mg thiamine 2 mg Ca pantothenate 0.5 mg agar 20 g pH 7 0

killed through the use of alcohol in dissolving the nystatin and therefore prevention of the outgrowth of most remaining viable bacteria may be aided by incorporation of other antibiotics for example tetracycline into the medium

Nystatin in solution is partially adsorbed by various types of bacterial filters but if quantitative recovery is not important alcoholic solutions may be sterilized by filtration through UF fritted glass filters

Alcoholic solutions of nystatin stored at room temperature will show measurable losses in activity over a period of one week but for practical purposes these losses may not be troublesome over a four day period At icebox temperature (*ca* 4°C) such solutions remain stable for much longer periods of time with no evident loss over a period of a month Both nystatin powders and solutions are best stored in the dark and in the cold Because of the instability in the presence of pH changes it is well to remember that acid media (below pH 6 0) and basic media (above pH 7 5) should be avoided At 37°C nystatin even in neutral media will begin to decompose in a day or two and in those cases where nystatin is being used to hold down growth of fungi and where there is a possibility of continued exposure to contaminating fungi it may be necessary to replenish the antibiotic periodically

FACTORS AFFECTING IN VITRO ACTIVITY OF NYSTATIN

Hickey[1] has reported that the activity of the antifungal antibiotic Ascosin is markedly lowered in the presence of unsaturated fatty acids such as oleic, linoleic and linolenic but not by saturated straight chain fatty acids This author also found that Tween 80 and to a lesser degree yeast and liver extracts showed this action We have noted that qualitatively nystatin responds similarly to the unsaturated fatty acids but apparently to an appreciably lesser degree than does Ascosin Tween 80 casamino acids and yeast extract on the other hand had no effect on the action of nystatin These findings are shown in Table 1

In this study to a synthetic medium * supplemented with 0 5

* The medium used is a modification of that described by Williams et al [2] sucrose 20 g $(NH_4)_2SO_4$ 5 g KH_2PO_4 2 g $MgSO_4 \cdot 7H_2O$ 250 mg $CaCl_2 \cdot 2H_2O$

On the other hand if *Saccharomyces cerevisiae* is used as the test organism this decrease in zone size does not occur This may indicate that tetracycline simply increases the rate of growth of *C albicans* resulting in some decrease in the zone of inhibition caused by nystatin It is interesting to note that if a tube test is employed normal results are obtained in the assay of nystatin whether or not

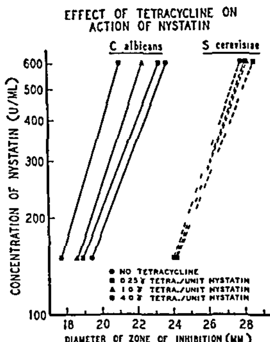


FIGURE 2

tetracycline is present The problem of the effect of other antibiotics on the activity of nystatin under various test conditions is still under active study in our laboratories

FUNGICIDAL ACTION

Hazen and Brown [4] demonstrated that nystatin showed fungicidal action against *Cryptococcus neoformans* We have observed similar results with *C albicans* With a strain of the latter for which the minimal inhibitory concentration of nystatin is *ca* 4 to

the case As already noted, interference with the activity of nystatin by the unsaturated fatty acids was considerably less than that noted for Ascosin by Hickey As regards Hickey's findings that Tween 80 interfered with Ascosin's action one might raise the question of whether his sample of Tween 80 was completely free of oleic acid resulting from hydrolysis

Several years ago Raubitscheck Acker and Waksman [8] described an antifungal antibiotic obtained from *Streptomyces aureus* which

TABLE 2

STABILITY OF MIXTURES * OF NYSTATIN AND FATTY ACIDS

Compound	Potency of Controls u/cc	% Loss in Activity
nystatin	300	44
nystatin + palmitate	336	46
nystatin + oleate	277	100
nystatin + undecenoate	294	100

*Incubated in air at 40°C for 3 weeks

they reported to be similar to nystatin (then called fungicidin) In passing these authors noted that 1/100 M cysteine hydrochloride caused complete reversal of activity of their antibiotic in agar streak plates Their test organism was not given Using *C albicans* we find this concentration of cysteine hydrochloride inhibitory in both agar or broth but at a final concentration of 300 γ/ml this amino acid causes a four fold increase in the minimal inhibiting concentration (MIC) of nystatin

In certain types of commercial formulations of nystatin one may be faced with the problem of assaying this antibiotic in the presence of other antibiotics The second antibiotic may commonly be tetracycline and when this is the case very misleading results are obtained in agar diffusion tests using *C albicans* as the test organism This is shown in Figure 2 Here increasing concentrations of tetracycline cause decreasing zones of inhibition of the Candida

DEVELOPMENT OF RESISTANCE

Repeated efforts to develop strains of *C albicans* resistant to nystatin by means of incorporation of the antibiotic in agar dilution tests broth dilution tests and gradient plates have not been successful With prolonged incubation the presence of viable cells

TABLE 3

EFFECT OF PROLONGED INCUBATION AT 37 C ON COLONY COUNTS OF *CANDIDA ALBICANS* ON AGAR PLATES CONTAINING NYSTATIN

Concentration of Nystatin	Duration of Incubation at 37°C (Days) 1	2	3	4	5	6	7	8
units/ml	Colony Count/Petri Plate x 10^5							
0	262	267	267					
1.0	298	302	305					
2.0	242	242	242					
4.0	265	265	265					
8.0	240*	240	240	240				
16.0	0	many*	119	119				
32.0	0	0	0	0.054		0.061		
48.0	0	0	0	0		0	0	0
64.0	0	0	0	0		0	0	0

*Microcolonies, too small to count with accuracy

may be demonstrable but on reisolation and retest they have invariably been found to be as sensitive as the parent Judging from the manner in which colonies of *C albicans* gradually appear on plates containing nystatin with prolonged incubation at 37 C and because of the constant sensitivity of the organisms reisolated from these colonies one is led to the conclusion that outgrowth is only possible because the nystatin has become inactivated in the medium during the incubation The rate of appearance of *C albicans* colonies on agar plates containing various concentrations of nystatin is shown in Table 3

The rate of decomposition of nystatin in culture medium at 37 C can be demonstrated in another quite simple fashion Here

8 units/ml rapid killing is observed over a six hour period of exposure to 32 units/ml at 37°C This is shown in Figure 3

It can be seen that the cell count dropped rapidly from 10^5 to 10^2 cells per milliliter (or 99 9 per cent kill) However at this concentration of antibiotic *ca* 0 1 per cent of the cells were still viable even after twenty four hours exposure The surviving cells were

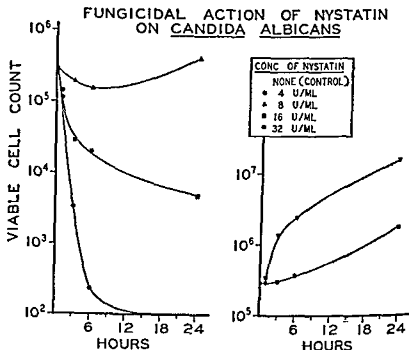


FIGURE 3

found to be as sensitive to nystatin as the parent culture The explanation for the survival of these cells therefore was of necessity sought elsewhere than in the development of resistance A natural question was whether nystatin became inactivated in culture medium at 37°C Since this proved to be the case it could also explain the apparently peculiar behavior of lower concentrations of nystatin for example 8 units/ml in which the Candida cell count dropped somewhat over the first six hours of exposure and then began to rise again a situation usually attributed to the development of resistance

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the antibiotic was incorporated in agar pour plates and incubated at 37 C a new sector of each plate being seeded with *C albicans* each day of incubation The apparent increase in minimal inhibitory concentration of nystatin for the test organism shown in Table 4 *is indicative of the rate of decomposition*

At 5°C nystatin is apparently much more stable in culture media From the results of experiments going on at present in our

TABLE 4
RATE OF DECOMPOSITION OF NYSTATIN IN PENASSAY AGAR PLATES

Time of Incubation Before Inoculation days	Apparent MIC for Candida albicans units/ml
0	8
1	12
2	16
3	40-48
4	>48

laboratories it appears that the antibiotic shows little loss in activity for a period of at least ten days when incorporated in agar plates and stored at icebox temperature These studies will be reported in fuller detail elsewhere

Preliminary efforts to obtain nystatin resistant strains of *Microsporum canis Trichophyton mentagrophytes*, and *Penicillium notatum* have thus far failed but several strains of *Ceratostomella ulmi* (the plant pathogen which causes Dutch elm disease) have been isolated which are two to four fold more resistant than the parent culture The question of development of resistance to nystatin obviously remains open and is being actively pursued in our laboratories

BIOASSAY OF NYSTATIN

Pagano and Stander describe in the following paper the details of a procedure for assaying nystatin in body fluids However solu

tions of nystatin in menstra other than body fluids for example in water or in organic solvents are assayed in our laboratories in a different manner The latter procedure will be described in detail elsewhere but may be summarized as follows ½ inch paper disks are moistened with various dilutions of the unknown solutions of nystatin and placed on large agar plates (3-quart pyrex baking dishes seeded with *C albicans*) according to a predetermined de sign which allows for statistical analyses Appropriate controls are included on each plate The plates are incubated at 37 C for six teen to seventeen hours and then photographed on autopositive pa per in order to provide a permanent record The zones of inhibi tion are then measured and by comparing the average zones for unknowns and standards in a four point design (low and high con centrations of unknown and standards respectively) the potency of the unknowns may be determined In this test the 95 per cent confidence limits for a single test are approximately ± 10 per cent

When it is necessary to assay nystatin in the presence of tetra cycline *Saccharomyces cerevisiae* (S C #1600) is substituted for *C albicans* as the test organism and Tween 80 is omitted from the medium These changes provide an assay procedure in which no distortion caused by the presence of tetracycline occurs It is how ever necessary to continue the use of *C albicans* in the assay of such experimental materials as nystatin fermentation broths be cause a second antifungal factor is also formed (see Hazen and Brown 1951) which is active against *S cerevisiae* but not against *C albicans*

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J F PAGANO and H STANDER

Bioassay of Nystatin (Mycostatin) in Body Fluids

The growing use of nystatin (Mycostatin *) as a therapeutic agent in the treatment of fungal infections [1 5] has necessitated the development of quantitative assay procedures for the antifungal agent in various body fluids Tarbet et al [6] have estimated the concentration of nystatin in blood by measuring the effect of the drug on the inhibition of germination of blastospores of *Candida tropicalis* A tube dilution procedure was reported by Gold et al [7] to be useful in the assay of blood samples

The procedure to be described is a 1 ml volume tube dilution bioassay having the properties of sensitivity simplicity and reproducibility these properties having been established by continued use of the assay in connection with the experimental pharmacology of nystatin [8]

MATERIALS AND METHODS

The assay consists of four tube dilution procedures (Table 1) arranged to determine the concentration of nystatin in samples at different levels of potency using as the test organism the yeast *Saccharomyces cerevisiae*, Squibb #1600 The organism is grown at 37°C on agar slants of a nutrient medium having the following composition

Tryptone	5 gm
Yeast Extract	3 gm
Malt extract	3 gm
Glucose	10 gm
Agar	15 gm
Distilled H_2O to 1000 ml	
Sterilized at 15 pounds for 15 minutes	

The culture on agar slants is stable at 50°C for at least one month

* Mycostatin is the E. R Squibb & Sons trade name for nystatin

TABLE 1

FOUR-TUBE DILUTION TEST PROCEDURES

50% Test (Tube)	1	2	3	4	5	6	7	8	9	10
Sample (ml)	.5	.4	.32	.26	.21	.17	14	.11	.09	.07
Normal Body Fluid (ml)	.0	.1	.18	.24	.29	.33	36	.39	.41	.43
Medium (ml)	5	.5	.5	5	.5	.5	.5	.5	5	.5
Sample dilution	1/2	1/2.5	1/3.12	1/3.85	1/4 76	1/5.89	1/7.15	1/9 1	1/11.1	1/14 3

30% Test (Tube)	1	2	3	4	5	6	7	8	9
Sample (ml)	.3	.21	.17	.14	.11	.09	.07	.056	.043
Normal Body Fluid (ml)	.0	.09	13	.16	19	.21	.23	.244	.257
Medium (ml)	7	7	7	7	7	7	7	7	7
Sample dilution	1/3.3	1/4 75	1/5 9	1/7.15	1/9.1	1/11.1	1/14 3	1/17.8	1/23.3

15% Test (Tube)	1	2	3	4	5	6	7	8	9	10
Sample (ml)	.15	.12	.10	.08	.06	.05	.04	.032	.024	.019
Normal Body Fluid (ml)	.0	.03	.05	.07	.09	.10	.11	.118	.126	.131
Medium (ml)	.85	.85	.85	.85	.85	.85	.85	.85	.85	.85
Sample dilution	1/6 7	1/8.34	1/10	1/12.5	1/16 7	1/20	1/25	1/34.4	1/41.6	1/52.6

8% Test (Tube)	1	2	3	4	5	6	7	8	9
Sample (ml)	.08	.064	.052	.038	.029	.023	.018	.014	.011
Normal Body Fluid (ml)	.0	.016	.028	.042	.051	.057	.062	.066	.069
Medium (ml)	92	92	92	92	92	92	92	92	92
Sample dilution	1/12.5	1/15.6	1/19.3	1/26.4	1/34.5	1/43.5	1/55.6	1/71.5	1/91.0

In preparing inoculum for the test a 250 ml flask containing 80 ml of the above medium from which the agar has been omitted is inoculated with the yeast culture taken from an agar slant The culture flask is incubated at 37°C (static) for seventeen hours This inoculum culture can be used for one week if stored at 5 C

The test medium is prepared with Difco Penassay Broth made

up at triple strength by dissolving 52 5 gm of the powder as supplied into 1000 ml of distilled water and sterilizing in 250 ml lots for fifteen minutes at 15 pounds pressure The final glucose concentration is raised from 0 3 to 1 3 per cent by the separate addition of a concentrated sterile glucose solution To depress the growth of bacterial contaminants which may be introduced with the sample to be tested the bulk medium is dosed with 10 units each of penicillin G and streptomycin HCl per milliliter of medium

To increase the sensitivity of the test a concentration of nystatin is added to the test medium equivalent to one half the expected minimal inhibiting concentration (MIC) In addition to giving a 100 per cent increase in sensitivity the test can be performed with *smaller sample volumes*

The inoculation of the test medium is made by adding 1 ml of inoculum to 100 ml of the test medium The inoculated test medium is dispensed into clear sterile 13 × 100 mm culture tubes in the amounts specified in the table of test procedures (Table 1) After addition of the appropriate standards and samples the tubes are incubated at 25° ± 0 5°C for seventeen hours

The maintenance and preparation of the nystatin standard * constitutes one of the most important steps in obtaining reproducible assay results The nystatin standard should be stored at 5°C in a desiccator containing a suitable drying agent In preparing the standard a weighing of 10 mg of the solid is dissolved in 20 cc of 70 per cent n propanol by acidifying to pH 3 0 with 0 1N HCl When complete solution has been achieved the solution is neutralized to pH 6 8–7 2 with 0 1N NaOH The neutralized solution is stable for at least one week at 5°C

A concentration of n propanol above 1 per cent in the test tubes is inhibitory to the yeast The standard should therefore be diluted to a concentration of approximately 200–300 units/ml in 70 per cent n propanol and the dilution to the desired test level made in the body fluid involved in the test

Determination of the nystatin level in blood is carried out by collecting the whole blood in a vessel containing sodium citrate (final concentration of 0 5 per cent) and centrifuging the sample for

* The nystatin unit is based on the value of 1000 units/mg assigned to the master standard HA224AX maintained at E R. Squibb & Sons

fifteen minutes at 2000 rpm After decantation the plasma is ready for assay Due to partial loss of nystatin activity during the process of sample preparation it is necessary to include controls to meas ure and correct for the magnitude of this loss Simultaneously with the drawing of the blood sample an accurately prepared solution of nystatin is diluted in normal whole blood to the level expected in the sample This control should receive the same treatment as the test sample that is centrifugation temperature time of expose etc The test sample potencies should then be adjusted upward by the percentage loss in activity found in the control

An acetone solvent extraction procedure is utilized in the deter mination of nystatin in feces Equal weights of feces and water are mixed to a smooth consistency in a Waring blender One part of the mixture is diluted with 50 parts of water and the suspension is mixed thoroughly An aliquot of the dilution is added to an equal portion of acetone After thorough mixing the sample is centri fuged at 2500 rpm for twenty minutes The supernate is decanted into a graduated centrifuge tube and evaporated to half volume to remove the acetone by directing an air stream to the surface of the sample The diluting fluid used in the assay of fecal samples is pre pared by making a similar extract of normal feces In addition to its function as an extracting solvent acetone serves to eliminate the bacterial contaminants in the test sample which would other wise seriously interfere with the assay An accurately weighed and diluted control should be included to measure extraction efficiency and loss of activity due to manipulations

The preparation of urine for assay requires acetone treatment to reduce the problem of contamination of the test Equal volumes of urine and acetone are mixed together and allowed to stand for several minutes The acetone is then removed by use of an air stream as described above and the sample is ready for assay A con trol of a known concentration of nystatin in urine treated in the same manner as the sample is essential

The inclusion of a control consisting of normal body fluid at a level comparable to that used in the test is necessary to determine the effect if any of the normal body fluid on the growth of the test organism

In the design of the assay a 20 per cent difference in concentra tion of sample exists between tubes With changes in the nystatin

concentrations of this magnitude several tubes will contain partial inhibition of the test organism To facilitate the reading and to in crease the sensitivity of the test, an illuminated reading box (Fig 1) is used Essentially the end point in the partially inhibited group of tubes is selected on the basis of a degree of turbidity in the tube which just obscures the readability of a group of numbers letters or words The tube preceding this tube is considered the end point from which the minimal inhibiting concentration (MIC) is calculated The calculation of a potency is made as fol lows

1 The MIC of the nystatin standard is obtained

$$\frac{\text{Concentration of nystatin standard solution in units/ml}}{\text{Reciprocal of the dilution of the standard required to obtain the end point}} = \text{MIC of nystatin standard in units/ml}$$

2 The potency of the sample or control is calculated

$$\text{MIC of nystatin standard in units/ml} \times \text{Reciprocal of the dilution of the sample or control solution required for the end point} = \text{Potency of sample or control solution in units/ml}$$

RESULTS

The reproducibility of the assay results was estimated from ex perimental data obtained with a group of sixteen urine samples The urine samples were assayed using the 8 per cent test (Table 2) They were then frozen overnight and reassayed the following day The reproducibility of results was estimated from the differ ence in sample dilution required to inhibit the test organism on day 1 as compared to day 2 Calculation of the standard deviation using this difference (Table 2) was based on the method described by Duncan [9]

$$\sigma' = \frac{\bar{R}}{d_2}$$

Where σ' is the standard deviation $\bar{R}$ is the average difference or average of the ranges between samples on day 1 and day 2 and d_2

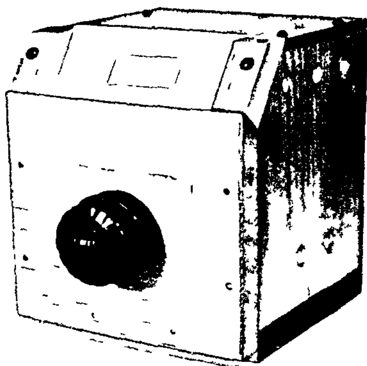


FIGURE 1
Illuminated reading box for assay

is a computational factor which increases with the increase in the number of samples

The results (Table 2) indicate that the standard deviation of a single determination is of the order of magnitude of 15 per cent The error of the test can be decreased by increasing the number of independent assays conducted on each sample The magnitude of

TABLE 2

BETWEEN-DAY REPRODUCIBILITY OF 1-CC. TUBE DILUTION BIOASSAY OF NYSTATIN

Sample	*Reciprocal of the Minimum Inhibiting Dilution (MID) Day 1/Day 2*	*Ratio of the* $\frac{High\ MID}{Low\ MID}$ *Expressed in Logarithms*
1	55 6/55 6	0 000
2	34 5/34 5	0 000
3	19 3/19 3	0 000
4	43 5/71 5	0 215
5	55 6/55 6	0 000
6	43 5/55 6	0 107
7	43 5/43 5	0 000
8	19 3/19 3	0 000
9	34 5/26 4	0 117
10	71 5/71 5	0 000
11	12 5/12 5	0 000
12	12 5/12 5	0 000
13	19 3/15 6	0 093
14	55 6/43 5	0 107
15	26 4/12 5	0 324
16	26 4/19 3	0 137
		$\bar{R}$ 0 069

$$= \frac{\bar{R}}{d_2}† = \frac{0\ 069}{1\ 128} = 0\ 061 = 15\%$$

Calculated from test data of urine samples
† From Duncan

the decrease in the standard deviation can be calculated by dividing the standard deviation for a single determination by the square root of the number of replicates

The nystatin concentration required to produce the inhibition point is influenced by both the concentration of total solids in the medium and the concentration of body fluid used in the test The

effect of changes in the total solids of the medium on the MIC was studied Several concentrations of Difco Penassay Broth were prepared and their effect on the MIC in the 30 per cent plasma test was obtained

It is apparent from the data in Table 3 that increasing the total solids in the test medium decreases the concentration of nystatin required to inhibit the assay organism What is not apparent from the data presented is that at a higher level of solids the growth of the yeast is more vigorous and the contrast in turbidity between

TABLE 3

EFFECT OF VARIOUS CONCENTRATIONS OF DIFCO PENASSAY BROTH MEDIUM ON MIC OF NYSTATIN IN DOG PLASMA

Difco Penassay Broth	*MIC in units/ml 30% Plasma Test*
Half strength	1 1
Regular strength *	0 9
Double strength	0 8
Triple strength	0 7

* Made by adding 17 5 gm of Difco Penassay Broth powder to 1000 ml of distilled water

the tubes at or near the end point is more marked than that obtained in the less concentrated media Hence the increased concentration of solids in the medium gives both an increase in sensitivity and in readability of the test

The introduction of sample plasma into the test results in a marked shift in the MIC of nystatin as evidenced by the data in Table 4 As the concentration of plasma is increased from 0 to 50 per cent the MIC increases Therefore the necessity for equilibration of each tube of the test to a constant level of plasma or other sample fluid becomes obvious

It is frequently necessary to hold samples for a period of hours or days prior to performing the assay The delay between the time of collection of the sample and its assay may under certain conditions lead to considerable loss in activity The controls which have been discussed above permit the estimation of the loss and correction of the observed potency figures

The stability of nystatin in dog urine at various pH s has been

TABLE 4

EFFECT OF DOG AND MOUSE PLASMA ON MIC OF NYSTATIN

Dog Plasma Concentration	*MIC units/ml*
%	
0	1 0
8	1 0
15	1 1
30	2 0
50	2 0
Mouse Plasma Concentration	
%	
8	0 6
15	0 7
30	1 0
50	1 7

determined (Table 5) The data indicate that no loss in activity occurs for a period of one hour at room temperature It has been previously shown that if normal urine samples containing nystatin are frozen for twenty four hours there is no loss of nystatin activity (Table 2)

The stability of nystatin in dog plasma and in water at room temperature was investigated (Table 6) Activity losses occurred in both samples over a period of time However no loss of activity was detected within the first hour Additional data have indicated that refrigeration of plasma samples at 5 C overnight resulted in a greater than 50 per cent loss in nystatin activity Plasma samples kept in the frozen state for twenty four hours showed no loss in activity

TABLE 5

STABILITY OF NYSTATIN IN DOG URINE FOR ONE HOUR AT pH 6 7 AND 8

pH	*MIC: Nystatin units/ml*	
	Initial	*One Hour*
6	0 93	0 93
7	0 93	0 93
8	0 74	0 74

TABLE 6

STABILITY OF NYSTATIN IN WATER AND DOG PLASMA AT 25 C

Time Intervals (hrs)	*Per Cent Loss in Activity*	
	Plasma	*H_2O*
0	0	0
1	0	0
2	16 5	0
3 5	16 5	16 5
5	37 5	16 5
6	50 0	37 5
19	70 0	—

The method of using solvent sterilization of samples of body fluid was employed after experimental data indicated that loss of nystatin activity occurred on passage of small volumes of a dilute solution of the antifungal agent through bacterial filters of asbestos (Seitz) porcelain (Selas) sintered glass or plastic (millipore) Especially marked were the activity losses found on using the Seitz filters

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B A STEINBERG and W P JAMBOR
with the technical assistance of MRS A SORIANO

The Effect of Nystatin (Mycostatin) on Experimental Candidiasis in Mice and Embryonated Eggs

Screening techniques in the search for antifungal materials are similar to those employed for the study of antibacterial agents One problem confronting the investigator is the necessity of having adjuvants administered with infectious agents to promote pathogenesis in the experimental animal We believe that the use of such adjuvants adds another variable to the biologic system being studied and therefore should be avoided

Our object in planning this work was to determine whether a suitable screening test for antifungal agents was possible using embryonated eggs as the experimental tool Our data will show that the test utilizing *Candida albicans* and embryonated eggs is easily done and convenient to use We also attempted to devise a simple easily standardized test in mice The latter test was planned to eliminate the need of an adjuvant to enhance the infectivity of *C albicans* The secondary test we developed in mice is also of a simple nature and gives rapid accurate results Finally we demonstrated the activity of nystatin in both of these tests

Dr Erich Seligman of Beth Israel Hospital New York City supplied us with the culture of *C albicans* used in eggs We initiated infection by washing the growth from a forty eight hour culture on Sabouraud s dextrose agar and injecting various dilutions of the washings via the yolk sac into seven-day-old chick embryos The eggs died with average survival times of 63 to 129 hours and the LD_{50} was $10^{-7\,9}$

We harvested and pooled the yolk sacs of a number of dead embryos which had been infected with a 10^{-4} dilution of the cell sus

pension A 20 per cent suspension of this pool was then prepared in normal saline solution We ampuled the pool and stored half the ampules at 5°C and the remainder at −40°C in a dry ice stor age cabinet Our data (Table 1) demonstrate that the titer of the yolk sac pool was not adversely affected by storage at either tem perature for periods up to one year

The PD_{50} of nystatin against *C albicans* in embryonated eggs is approximately 4500 units/egg (Table 2) By using 6000 units/egg we succeeded in sterilizing 60 per cent of the eggs whereas all con trol eggs yielded rich cultures of *C albicans* We have demon strated that with 7500 units/egg it is possible to reduce the LD_{50} of *C albicans* for eggs by three full logs The activity of nystatin ap pears to be fungistatic rather than fungicidal in embryonated eggs

TABLE 1

INFECTIVITY TITRATIONS OF *CANDIDA ALBICANS* YOLK SAC SUSPENSION IN CHICK EMBRYOS AFTER DIFFERENT STORAGE CONDITIONS

Storage Temperature	*Length of Storage*	*Egg Infectivity LD_{50}**
Control	0	$10^{-7\ 4}$
− 40°C	1 month	$10^{-7\ 8}$
	2 months	$10^{-7\ 2}$
	6 months	$10^{-7\ 5}$
	12 months	$10^{-7\ 6}$
5°C	1 month	$10^{-7\ 8}$
	2 months	$10^{-7\ 4}$
	6 months	$10^{-7\ 2}$
	12 months	$10^{-6\ 7}$

* Reed and Muench method

TABLE 2

ACTIVITY OF NYSTATIN AGAINST *CANDIDA ALBICANS* IN EMBRYONATED EGGS

Mycostatin (units/egg)	*No of Eggs Treated*	*Average Survival Time*	*Survivors/ Tot l*
6000	32	230	17/25
3000	32	196	1/31
1500	32	122	0/29
Controls	32	98	0/25

For our studies in mice we used the CF #1 strain from Carworth Farms Dr Elizabeth Hazen kindly supplied the culture used in all these studies We grew the organism on Sabouraud s dextrose agar in Roux bottles for forty-eight hours at 37 C and washed the resultant growth off in 8 0 ml of physiologic saline solution The cell count was read off a standard curve after turbidity determinations

TABLE 3

NYSTATIN TESTED IN MICE USING DIMETHYLACETAMIDE AS A SOLVENT

Mice CF #1 males 21–24 gm
Infection intravenously with 0 5 ml of a *C albicans* suspension (2 × 10[6] cells/mouse)
Treatment b i d as indicated below for 2 days
Drug Preparation in DMA with a ratio of 1 10 of drug DMA

Preparation	*Units/ Dose*	*Units/ Day*	*Route*	*Average Survival Time (hrs)*	*Difference in Average Survival T m (hrs)*	*Survivors/ Total*	*No of Doses*
Saline	0 5 ml	—	s c	30	—	0/20	4
M3602-	1500	3000	s c	> 240	> 210	20/20	4
HV340-1F*	750	1500	s c	> 186	> 156	13/20	4
(free base)	375	750	s c	> 91	> 61	2/20	4
M3602-	1500	3000	s c	> 240	> 210	20/20	4
HV384-3	750	1500	s c	> 203	> 173	15/20	4
(crystalline)	375	750	s c	> 128	> 98	5/20	4

M3602 is the Squibb code number for nystatin

in a Bausch & Lomb colorimeter We adjusted the suspension to contain 4 × 10[6] cells/ml The mice were infected by injecting 0 5 ml of the suspension into the tail vein In this manner we were able to produce an infection that was uniformly fatal with an average survival time for mice of thirty to forty hours

We noted that early batches of nystatin did not possess all the activity they theoretically should have This we learned was a function of the physical state of the material being used When we suspended these preparations in water or gum acacia we were able to demonstrate only a low level of activity To show activity we found it necessary to solubilize the batches we were testing We

did this initially by using a mixture of calcium chloride and ethyl alcohol but later we found that N N dimethylacetamide (DMA) was more suitable With either solvent the addition of water produced a fine colloidal suspension Prepared in this way nystatin was extremely active against experimental candidiasis Under these conditions the PD_{50} for mice was approximately 750 units/mouse by the subcutaneous route (Table 3) We attempted to determine whether nystatin would eliminate *C albicans* from infected mice Prolonged treatment failed to clear the mice and small foci of infection remained in the kidneys (Table 4)

In another test we demonstrated that nystatin given subcutaneously would protect mice that had been infected intracranially We infer that nystatin is able to cross the blood brain barrier at least in the presence of infection Autopsy of the mice showed some minor renal lesions but the main infection was in the brain

TABLE 4

CANDIDA ALBICANS IN MOUSE TISSUES AFTER TREATMENT WITH NYSTATIN

Mice CF #1 males 20–23 gm
Infection intravenously with 0 5 ml of a suspension of *C albicans* (2×10^6 organisms/mouse)
Treatment b i d as indicated below by the subcutaneous route

Time Interval after Infection	*Cell Count in Ground Tissue*					
	Untreated Controls			*Nystatin 3000 units/mouse b i d for 2 days*		
	Heart	*Kidney*	*Spleen*	*Heart*	*Kidney*	*Spleen*
4 hours	51 000	290 000	92,000	68,750	110 000	74,000
1 day	3,700	1,465,000	14 350	13 900	1,750	3 250
2 days	D	E A	D	410	200	80
3 days				1 250	130	235
4 days				70	400	0
5 days				50	135	125
				Nystatin 3000 units/mouse b i d for 7 days		
3 days	D	E A	D	0	80	90
4 days				135	67	70
5 days				0	93	0
6 days				0	43	0
7 days				0	25	0

ELIZABETH L HAZEN, RACHEL BROWN,
and GEORGE N LITTLE

Moniliasis in Experimental Animals Prophylaxis and Therapy with Nystatin*

Experimental moniliasis induced in mice and rabbits in conjunction with tetracycline and its treatment with nystatin have been investigated

MICE

A rapidly fatal moniliasis was induced in mice by intraperitoneal injection of a mixture of *Candida albicans* (approximately 19 000 000 cells) and tetracycline hydrochloride (3 mg) *C albicans* was recovered from the heart s blood in 67 per cent of the animals and from the peritoneal cavity in 100 per cent Pinpoint white masses were present on the surface of the spleen and liver of 76 per cent of the mice This effect was markedly reduced either by subcutaneous injection (1 ml) of nystatin in aqueous suspension (2500 units/ml) two to three hours before or at the time of administration of the lethal mixture or by 100 units of nystatin suspended in the lethal mixture (Table 1) The infection bore scant if any resemblance to the clinical disease

RABBITS

Moniliasis of the mucous membrane of the mouth and mucocutaneous junctions having certain features of the clinical disease in man was induced in four rabbits receiving tetracycline The infection appearing usually on the third day after the oral administration of *C albicans* was characterized by a spreading inflammatory reaction covering the inner aspects of both lips the gingiva

E R Squibb & Sons Division of Olin Mathieson Chemical Corporation supplied the tetracycline (Steclin) and nystatin (Mycostatin) used in this investigation

TABLE 1

EFFECT OF NYSTATIN IN MICE INJECTED INTRAPERITONEALLY WITH A LETHAL MIXTURE OF *CANDIDA ALBICANS* AND TETRACYCLINE HCl

Material injected	*No in group*	*Nystatin**	*Deaths (days)*		*Survival %*	*Autopsy Results*			
						gross lesions†		*Isolation of C albicans*	
			1–5	*5–14*		+	−	*heart s blood*	*peritoneal cavity*
C albicans (19 000 000 cells) + 3 mg tetracycline HCl	9		9		0	6	3	6	9
Same	10	At time of infection	1		90		1	1	
Same	10	2–3 hrs before infection	2		80		2	1	1
Same	10	2 hrs after infection	5		50		4		4
Same + 100 units nystatin	10		3		70		3		
C albicans	10		5	10	0	8	1	5	4
Tetracycline HCl	5				100				
Nystatin 100 units	5				100				
Nystatin 2500 units (subcutaneously)	10				100				

*2500 units subcutaneously

† Very small white masses on surface of kidneys and/o pleen.

and the mucocutaneous junctions particularly at the commissures On the reddened surfaces a number of small white lesions appeared usually on the lower gingiva inner aspects of the lower lip and in some instances on the dorsum of the tongue near the tip A more constant and striking feature was the many small whitish lesions in the labial commissures where they coalesced and formed a whitish membranous film consisting chiefly of yeastlike cells and mycelium from which *C albicans* was isolated in pure form

Moniliasis was induced in the rabbits under the following conditions tetracycline hydrochloride (50 mg /kg) in capsules was administered orally twice daily for four days and *C albicans* (1 ml of packed cells) was given orally between the two doses on the third day After a rest period of two days tetracycline was administered twice daily for two more days Moniliasis was not demonstrated in two other rabbits which received the above regimen with the addition of nystatin (125 000 units/kg) to the capsules of tetracycline Nor did two rabbits which received *C albicans* alone develop infection

In one experiment two of three infected rabbits were given nystatin (125 000 units/kg) orally in honey or in capsules twice daily for three and a half days Marked improvement in the infection was noted between six and eighteen hours after the second dose and the infection had cleared after two days whereas the natural course of the disease in an untreated animal ran for twelve days The results of the experiment are summarized in Table 2

The effect of the antibiotics on the rabbit s normal intestinal bacterial flora and fungal flora following the ingestion of *C albicans* was studied in the five rabbits shown in Table 2 three receiving tetracycline one tetracycline nystatin combination and one no antibiotics Fecal material (1 gm) was macerated and divided into aliquot portions of 500 mg for the bacterial and fungal counts Exudates on swabs were obtained at the same time from their mouths for determination of *C albicans* growth The fecal material and mouth swabs were obtained before and forty eight hours after treatment with antibiotics twenty four and forty eight hours after administration of *C albicans* and thereafter at forty eight hour intervals for seven days

For the fungal counts the ground fecal material was suspended

TABLE 2

EFFECT OF NYSTATIN IN EXPERIMENTAL MONILIASIS ASSOCIATED WITH ORAL ADMINISTRATION OF TETRACYCLINE

Prior to infection			C albicans	After infection				
Rabbit No	Tetracycline*	Tetracycline + nystatin†		Tetracycline*	Tetracycline + nystatin†	Signs of infection	Nystatin‡	Outcome
717	+			+		Severe§		Improvement in 8 days recovery in 12 days
747	+			+		Severe	In honey	Improvement in 6 hours recovery in 2 days
735	+			+		Severe	In capsules	Improvement in 18 hours recovery in 2 days
691		+			+	None		
768						None		

* 50 mg./kg b.i.d. x 2 in capsules.

† 125 000 units/kg b.i.d. x 2 in capsules

‡ 125 000 units/kg, b i.d. x 3 1/2 days.

§ Loss of weight oral inflammation with whit sh patches on lips gingiva and labial commissures

Two other rabbits that received the same regimen of tet acycline but no C. albicans showed *no ill effects*.

in sterile salt solution containing 1000 units of penicillin 1000/μg of streptomycin and 100/μg of chlortetracycline per milliliter to give a 1 20 dilution from which further dilutions were prepared Dilutions were plated in 1 ml amounts on Sabouraud s glucose medium to which was added 1 ml of the antibiotic solution The plates were incubated for forty eight hours at 28 C The bacterial counts were made in the same manner with the exception that the dilution of 1 20 was made in a sterile salt solution containing 250 units/ml of nystatin and placed in the refrigerator for three hours before preparing the higher dilutions The plating medium was an extract agar to which 250 units/ml of nystatin were added the incubation period was twenty two to twenty four hours at 36 C The mouth swabs were streaked on Littman s agar and incubated for forty eight hours at 28 C

C albicans was not found in the fecal material or in the mouth exudates before administration of the *C albicans* The bacterial and *C albicans* counts indicated that the infection was established only in those rabbits receiving tetracycline The counts showed as a consequence a marked reduction in the normal bacterial intestinal flora at the time of administration of the infective dose of *C albicans* and an almost complete substitution of the bacterial flora with *C albicans* forty eight hours after ingestion of the fungus Mouth cultures also showed a heavy growth of *C albicans*

The rabbits receiving the combination of nystatin and tetracycline did not develop the infection although they showed a marked reduction in the normal intestinal bacterial flora at the time of ingestion of *C albicans* but forty eight hours after the infective dose there were fewer than twenty *C albicans* cells per gram of fecal material and there was only a scant growth of the micro-organism in the mouth cultures The rabbits receiving only the *C albicans* did not become infected and showed no reduction in the normal bacterial intestinal flora at the time of ingestion of *C albicans* Twenty four hours later the fungal count was less than twenty cells per gram of fecal material and there was only scanty growth of the fungus in the mouth cultures These results appear in Figure 1

The establishment of this infection apparently cannot be attributed to an enhancement of virulence in *C albicans* by tetracycline since no differences could be detected in the fatality rate in rabbits receiving lethal intravenous doses obtained from isolates from

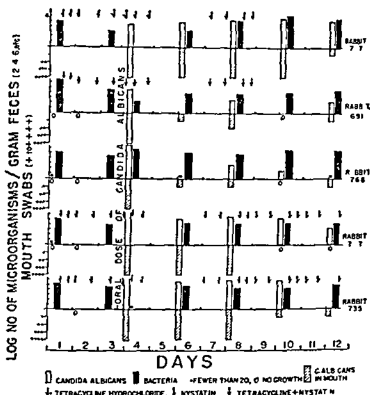


FIGURE 1

Therapeutic and prophylactic effects of nystatin on the intestinal bacterial and fungal flora and mouth fungal flora of five rabbits infected orally with *C albicans* in conjunction with tetracycline

an infected rabbit an untreated control rabbit and the stock isolate used in the experiments nor was there any difference in the gross pathology at autopsy

The establishment of the moniliasis however appeared to be directly related to the alteration of normal bacterial intestinal flora and apparently that of the mouth of the rabbit as a consequence of the tetracycline therapy

The results of these studies indicate that the rabbit is a suitable animal for the study of experimental moniliasis in conjunction with the broad spectrum antibiotics and for demonstration of the efficacy of nystatin as a prophylactic and therapeutic agent

BOHDAN DOBIAS

Cutaneous Moniliasis in Pediatrics Diagnosis and Treatment with Nystatin

Moniliasis of the skin is probably not as rare or always as benign as most commonly considered especially in young infants Apparently many cases of cutaneous moniliasis are frequently diagnosed as diaper rash infantile eczema or atopic dermatitis This clinical entity was almost simultaneously described for the first time by Beck [1] in 1910 and Ibrahim [2] in 1911

Very little accurate information is available to indicate how commonly *Candida albicans* plays a role as an etiologic agent of skin disorders Epstein [3] reported 4 cases of skin thrush among 270 cases of oral thrush In two years he saw 24 cases Maria Kaufmann Wolf [4] pointed out in 1915 that this mycosis of infants was in no way rare in Berlin In my experience it appears to be quite common Out of 70 patients presented here 61 were seen between November 1954 and April 1955 among our clinic patients and on the wards In this time about 1000 children under the age of twelve years were seen in the clinic

This study deals primarily with cutaneous moniliasis No patients with other lesions due to *C albicans* were included unless they revealed in addition evidence of skin moniliasis Only patients in whom the clinical diagnosis was confirmed by cultures of scrapings from skin lesions are dealt with in this series Thirteen patients were white and 57 were Negroes Most of them were from the lower economic group of the city population

Table 1 shows the age distribution at the onset of the disease

The lesions most commonly encountered in the newborn started as erythematous papules or tiny vesicles in the perianal or genital regions usually extending within one to seven days over the inner and posterior aspects of the upper thighs and lower abdomen to

the inguinal regions rarely also to the lower back (Fig 1) In most cases the lesions stopped spreading below the level of the umbilicus in a few the spread continued to the upper abdomen and chest and in 4 children the entire body was covered Soon after the appearance of papules or vesicles the lesions tended to become confluent The center was usually formed by a wide area of thin glistening pink or dusky red skin The borders were almost invariably sharp with a fringe of somewhat macerated epidermis

TABLE 1

AGE DISTRIBUTION AT ONSET

Age at Onset	*No of Patients*
0– 3 mos	40
3– 6 mos	5
6– 9 mos	8
9–12 mos	5
1– 2 yrs	4
2– 4 yrs	4
4– 6 yrs	3
Over 6 yrs	1
Total	70

Generally there were a few satellite lesions present (Fig 2) In intertriginous areas there was in some cases a variable degree of oozing

At the time when the rash in the diaper area appeared or a few days later some children developed similar lesions on the face A striking feature in nearly all Negro patients seen was a variable degree of transient depigmentation which was almost complete in 2 cases with very extensive lesions (Fig 3) Ten children with extensive involvement of the skin developed scaling lesions of the scalp in most cases with some transient loss of hair

Oral thrush was usually noticed several days before the onset of cutaneous manifestations In this series it was seen in 25 children

Whereas most of the newborn and young infants revealed a rather uniform pattern the morphology and localization of skin manifestations in older children were more varied Their lesions were generally deeper Joulia and Le Coulant[8] characterized this type as the chronic form of second childhood as compared to the

FIGURE 1

Early stage of cutaneous moniliasis with papular and vesicular lesions

FIGURE 2

Typical case of cutaneous moniliasis several days after the onset. Note peripheral desquamation and satellite lesions

FIGURE 3

Depigmentation of a Negro infant with cutaneous moniliasis Note satellite lesions and loss of pigment in axillary regions face and neck

FIGURE 4

Same patient as in Figure 1 after oral and topical nystatin

acute infantile form Some cases in this age group presented a continuation of the typical skin moniliasis of young infants

Two children presented erosio interdigitalis blastomycetica 5 children had paronychial swelling Angular stomatitis (perlèche) was observed in 4 cases Somewhat elevated scaling erythematous psoriasiform patches 1 to 5 mm in diameter were noted in 7 children scattered mostly on the face especially around the nose mouth and eyes

Unusual skin changes were found in a seven year-old boy with a few erythematous and vesiculopustular lesions of the face with slight scaling at the border of the erythema and slight facial swelling Bilateral blepharitis two rather large scaly scalp lesions with almost complete absence of hair and pustular scaling lesions on one hand were also present He had been having skin disorders since early childhood Two younger siblings had similar less pronounced lesions the mother had extensively involved skin on the extremities A similar familial incidence of moniliasis was found in six other families A history of maternal vaginitis was present in 16 cases

An even deeper type of lesion was seen in a thirteen month-old retarded girl with granulomatous moniliasis Her legs and arms were almost entirely covered with ulcerative confluent lesions composed of many small nodules and abscesses some of them discharging grayish pus The borders of the lesions were elevated slightly erythematous and sharply delineated Similar smaller lesions were present on the back of the neck and on the forehead The patient had a marked generalized lymphadenopathy

Lesions regarded as candidids were encountered several times They were mostly widespread papular or scarlatiniform eruptions or vesicular lesions on the hands

Several cases of cutaneous moniliasis presented symptoms of monilial lesions in other organs Bronchial or pulmonary moniliasis was suspected in 6 cases because of sudden bouts of dyspnea cyanosis and persistent cough All these patients had thrush lesions on the uvula and soft palate some also on the posterior pharyngeal wall This localization of thrush was unusual in other patients One patient also had a history of hemoptysis with prolonged expiration In one infant two months old with marked dyspnea and cyanosis who had x ray evidence of pulmonary infil

tration symptoms disappeared on treatment with nystatin alone within three days This was regarded as being somewhat suggestive of a monilial etiology Balanitis was encountered in 2 cases vulvovaginitis in 1 and conjunctivitis in 1 Many Candida organisms were cultured from a discharging ear of a two-year old girl with chronic bilateral otitis media treated for fifteen months intermittently with several antibiotics It is difficult to decide however whether otitis was primarily due to Candida or whether the growth of *C albicans* was enhanced by prolonged antibiotic treatment

Three cases of generalized moniliasis with death from alymphocytosis were reported in the literature (Glanzmann and Riniker [6] Donohue [7]) A similar patient was admitted to the Babies Hospital in Newark and transferred to another hospital where he died of alymphocytosis several weeks later It is interesting to note in this connection that in several reports (Lederer and Todd [8] Ebbs [9]) on patients with widespread infections due to *C albicans* the autopsy findings in some cases do not clearly explain the fatal outcome Most of the reported cases as well as the case admitted to the Babies Hospital presented oral and esophageal thrush refusal to take feedings vomiting and symptoms of severe toxemia with pallor and weak pulse No case of alymphocytosis has been described in a child without a Candida infection The theory that monilia infection was primary and that the fungus elaborated a toxin which resulted in an almost complete lysis of the lymphoid tissue considered unlikely by Glanzmann and Riniker [6] as well as Donohue [7] deserves further investigation especially in view of the recent work done by Salvin [10] who prepared a soluble endotoxin from the yeast cells of *C albicans* It would be interesting to investigate a possible action of this or a similar toxin on the lymphoid tissue

The importance of the knowledge of symptoms of cutaneous moniliasis deserves full appreciation since it can serve as supportive evidence in detecting some other more serious monilial lesions frequently associated with cutaneous moniliasis

Forty eight of the 70 patients in this series were treated with nystatin Thirty received nystatin by mouth alone 16 in both oral and topical form and 2 topically nystatin alone

In infants oral nystatin was usually given in doses of 100 000 units of the flavored powder in ½ ounce of formula milk or water three to five times daily In several instances crushed tablets were

used instead Nystatin ointment was applied two to four times daily to the skin lesions The mouths of 15 patients in whom skin lesions were associated with extensive oral thrush were swabbed with nystatin powder mixed with formula milk or glycerin One patient with chronic otitis media yielding *C albicans* was treated successfully by introducing crushed tablets in an irrigating solution concomitantly with oral medication

The best results were obtained in superficial cutaneous moniliasis of young infants Twenty one of 28 patients in this age group were cured 6 were improved and 1 was unimproved Improvement of skin lesions of this type was usually observed within two to seven days (Fig 4) Recurrence of symptoms in this category was observed in 5 cases but all of them showed complete clearance of lesions after the second course of treatment

All scalp lesions and perlèche in infants and older children responded to topical and oral treatment Symptoms of vulvovaginitis in a two-month old girl disappeared after five days of oral nystatin Four infants with oral and cutaneous moniliasis and *C albicans* in the stools experienced complete disappearance of the complicating diarrhea which was probably due to gastrointestinal moniliasis Slower but definite improvement was usually observed in older children with chronic lichenified or hyperkeratotic lesions A three year old girl with extensive monilial skin lesions that first started at the age of two months was cured after three months on oral and topical nystatin

No side effects including diarrhea reported by some workers [11, 12] were noted in any of the patients in this series Oral treatment of pediatric patients with cutaneous moniliasis has been sufficient in some cases even though relatively little absorption from the intestine occurs The concomitant use of nystatin ointment appears to be the treatment of choice In my experience the treatment should be continued for several days after complete disappearance of the symptoms in order to prevent recurrences

The use of nystatin in pregnant women with vaginal moniliasis one of the important sources of infantile infection could probably prevent many cases of neonatal moniliasis

The results of treatment in 48 patients are summarized in Table 2

In conclusion I should like to emphasize that in my opinion

Table 2
RESULTS OF TREATMENT

Route	*No of Patients Treated*	*Cured*	*Improved*	*Not Improved*
Oral only	30	12	18	0
Oral and topical	16	10	5	1
Topical only	2	2	0	0
Totals	48	24	23	1

nystatin is a very useful substance in the treatment of cutaneous moniliasis in pediatrics especially in superficial infections of young infants and also in other forms of the disease

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Therapeutic Activity of Nystatin (Mycostatin) in Candida Infections*

The clinical manifestations of Candida (Monilia) infections have been known for many years Oral thrush considered as a relatively mild *C albicans* infection occurring especially in debilitated children and in the elderly may become a more serious condition by extension to the pharynx bronchi or esophagus or it may even invade the blood stream and reach the kidneys brain and other organs Trousseau and Parrot [1] in France have observed and described such cases as having occurred in high incidence in nurseries more than one hundred years ago

With the introduction of intensive antibiotic therapy there has been a considerable increase not only in the incidence of Candida infections but also in the severity of the infections observed in infancy as well as in childhood and in adult age

In France Lelong et al [2] and recently Debré et al [3] observed and described fatal cases of generalized thrush in infants complicating prolonged therapy with broad spectrum antibiotics The increased incidence of Candida infections is observed also in adults in gynecology and obstetrics [4] in dermatology [5] and in hematology [6]

The need for an effective agent to control the complications of Candida was satisfied by nystatin the antifungal antibiotic discovered by Hazen and Brown [7] This antibiotic was effective in experimental moniliasis in mice [8, 9] and decreased the yeast flora of animals and man [9, 10]

Additional experiments in the Pasteur Institute showed the antifungal effect of nystatin in vitro and in animals and the lack of toxicity after oral administration This made it possible to apply nystatin in human cases particularly in infants and children

The material used in this work was suppl ed through the courtesy of the Squibb Inst tute for Med cal Research New Brunswick New Jersey

where a generalized *C albicans* may be particularly dangerous [4, 11] After the favorable clinical results obtained in the first cases treated and presented by Debré et al [3] at the pediatric meeting in Paris in June 1954 numerous other cases of generalized and localized moniliasis *were treated with nystatin The mycologic studies and the* multiple controls of these cases were made in the Laboratory of Mycology of the Pasteur Institute Recently Grupper [6] also obtained favorable results in cutaneous moniliasis treated with nystatin

This report summarizes our data concerning the effect of nystatin in vitro on various strains of Candida in experimental infection with *C albicans* in rabbits and in 43 cases of human moniliasis

IN VITRO

Nystatin inhibited the growth of all species of Candida studied (fifty five strains) the minimal concentration for a total inhibition for forty eight hours on a semi synthetic agar medium was between 6 38 and 25 56 units (Table 1) lower values were obtained in liquid medium Nystatin is not only fungistatic but also fungicidal

TABLE 1

SENSITIVITY TO NYSTATIN OF VARIOUS STRAINS OF CANDIDA AND GEOTRICHUM ON AGAR MEDIUM

SPECIES	NUMBER OF STRAINS STUDIED	NYSTATIN * µg/cc	units/cc
C albicans	3	3 12	6 38
	28	6 25	12 78
	4	12 50	25,5b
C tropicalis	4	6,25	12,78
C pseudotropicalis	3	6 25	12 78
	6	12 50	25 56
C krusei	2	12 50	25,56
C parakrusei	5	12 50	25 56
Geotrichum sp	2	6 25	12 78
	3	12 50	25 56

* Minimal concentration for total inhibition for 48 hours on a semi-synthetic medium

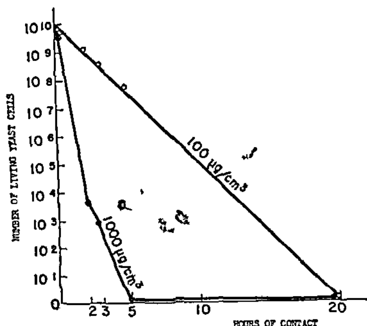


FIGURE 1

Fungicidal activity of nystatin on *Candida albicans* strain MIC.

when sufficiently high concentrations are used (Fig 1) No case of resistance to nystatin has been observed as yet The sensitivity of *C albicans* to nystatin was unchanged in the several cases in which relapse occurred

IN VIVO

EXPERIMENTAL INFECTION OF RABBITS

The mortality of rabbits infected intravenously with *C albicans* was reduced to 62 per cent by subcutaneous administration of 40 mg (80 000 units) daily for five days With oral administration no effect on the mortality rate was observed due to poor absorption of nystatin

The experimental infection of the digestive tract which compares better with the natural human infection was studied in rabbits and it was proved that the administration of nystatin produces

a considerable reduction of the number of yeasts in the intestinal tract When nystatin was discontinued early *C albicans* reappeared in the stools

HUMAN CASES

Forty three cases of moniliasis were studied in infants (24 cases) children (12 cases) and adults (7 cases) The laboratory diagnosis included in all cases microscopic examination and cultures from the oral mucosa stools urine and sometimes from pus as well as from blood In all these cases *C albicans* was identified

Of the 43 cases studied 13 were cases of generalized moniliasis with clinical manifestations appearing as thrush diarrhea vulvovaginitis and multiple abscesses There were 25 cases of localized digestive moniliasis (thrush diarrhea or abdominal distress) 2 of cutaneous moniliasis with onychia and paronychia and 3 with moniliasis of different localizations (Table 2) All these cases had been intensively treated with the usual antibacterial antibiotics in various combinations prior to the use of nystatin The clinical manifestations such as thrush were present from several days to several weeks and the ordinary therapy was without effect

Nystatin was remarkably effective A rapid recovery of clinical moniliasis was obtained in all cases and the organisms decreased in number or disappeared completely from the mouth stool urine or blood Nystatin was administered in powder form mixed with food in doses of 0 1 gm per kilogram weight daily (200 000 units) for infants and children under 5 kg and for others between 0 5 and 1 gm (1 to 2 million units) daily The powder form is the most adequate form for the treatment of digestive moniliasis because generally the whole gastrointestinal tract is involved in such cases beginning from the oral cavity The best results were obtained in cases of *C albicans* infection complicating antibacterial therapy where only four to eight days were needed to obtain clinical healing and sterilization In many cases clinical relapse did not occur even though Candida organisms reappeared A few relapses occurred (8 in 43 cases) and these relapses were seen [12] chiefly in premature infants (4 of 8 studied) in which the appearance of thrush was unrelated to antibiotic therapy (Tables 3 and 4)

The chronic cases as in the cutaneous cases with involvement of the nails require a more prolonged oral and topical treatment In

TABLE 2

CANDIDA ALBICANS INFECTIONS TREATED WITH NYSTATIN

I GENERALIZED MONILIASIS: 13 cases (4 infants 7 children 2 adults)			
CLINICAL MANIFESTATIONS OF MONILIASIS	NO OF CASES	ISOLATION OF C ALBICANS	NO OF CASES
THRUSH	13	MOUTH	13
DIARRHEA	5	STOOL	13
VULVOVAGINITIS	2	URINE	13
GLUTEAL ABSCESS	2	BLOOD	3
PLEURAL ABSCESS	1		
PRIMARY DISEASE		ANTIBIOTIC TREATMENT PRIOR TO APPEARANCE OF MONILIASIS	
STAPHYLOCOCCOSIS	6	PENICILLIN	11
LEUKEMIA	4	STREPTOMYCIN	10
TOXICOSIS	2	CHLORAMPHENICOL	6
OTOPHARYNGITIS	1	TERRAMYCIN	5
		AUREOMYCIN	4
		ERYTHROMYCIN	3
II LOCALIZED MONILIASIS OF DIGESTIVE TRACT. THRUSH DIARRHEA ABDOMINAL DISTRESS			
25 CASES: 8 PREMATURE INFANTS, 12 INFANTS, 3 CHILDREN, 2 ADULTS			
III CUTANEOUS CHRONIC MONILIASIS WITH PARONYCHIA AND ONYCHIA.			
2 CASES: 1 CHILD- 3 years, 1 WOMAN-70 years			
IV VARIOUS LOCALIZED MONILIASIS.			
3 CASES: Vaginitis (adult), Pharyngitis and chronic bronchitis (adult), Urinary complication (infant)			

tensive search is advisable to establish the primary condition (avitaminosis endocrine conditions) responsible for the *C albicans* involvement

The lack of toxicity of nystatin is remarkable It is perfectly tolerated by infants even those in very poor condition

TABLE 3

ACTION OF NYSTATIN IN SEVERAL GENERALIZED CASES OF MONILIASIS*

CASE NO	PRIMARY INFECTION TREATED WITH ANTIBIOTICS AGE & WEIGHT	SYMPTOMS OF DISEASE	ISOLATION OF C ALBICANS BEFORE AND AFTER TREATMENT					NYSTATIN ORALLY IN POWDER FORM DAILY DOSE		NO OF DAYS	CLINICAL COURSE OF MONILIASIS
			MOUTH	STOOL	URINE	BLOOD	ABCESS	MG	UNITS		
1	ACUTE LEUKEMIA 8 YRS., 66 LB	SEPTICEMIA THRUSH VULVITIS	+ ± 0	+ ±	+ 0	+ 0		800 800	1,120,000 1,120,000	4 8	RECOVERY IN 3 DAYS
2	STAPHYLOCOCCOSIS 2 YRS, 16 LBS	SEPTICEMIA THRUSH	+ 0	+ 0	+ 0	+		700	980,000	3	RECOVERY IN 24 HOURS
3	STAPHYLOCOCCOSIS 6 WKS, 8½ LBS	THRUSH GLUTEAL ABCESS WEIGHT LOSS	+ + 0	+ + 0	+ + 0	+ + 0	+ + 0	1,000 500	1,400,000 700,000	1 3	RECOVERY IN 4 DAYS
4	STAPHYLOCOCCOSIS 18 MOS, 18 LBS	THRUSH DIARRHEA GLUTEAL ABCESS TOXICOSIS	+ 0	+ 0	+ 0	0	+ 0	800	1,120,000	4	RECOVERY IN 3 DAYS
5	STAPHYLOCOCCOSIS 18 MOS., 22 LBS	THRUSH, VULVITIS PLEURAL ABCESS RASH	+ 0	+ 0	+ 0		+ 0	1,000 500	1,400 000 700,000	1 4	RAPID RECOVERY RELAPSE AFTER 15 DAYS
6	OTOPHARYNGITIS 5 MOS, 12½ LBS	THRUSH DIARRHEA HEMATURIA	+ + 0	+ 0 0	+ 0 0			500 500	700,000 700,000	4 4	RECOVERY IN 4 DAYS
13	ACUTE LEUKEMIA, 58 YRS	THRUSH DYSPHAGIA DIARRHEA	+ ± GEOTR 0	+ ± 0	+ 0 0	0 0		500 1,000	700,000 1,400 000	3 3	RECOVERY IN 4 DAYS

For additional detail see references 3, 6 and 11

TABLE 4

ACTION OF NYSTATIN IN SEVERAL LOCALIZED MONILIASIS OF DIGESTIVE TRACT IN CHILDREN UNDER 8 MONTHS[11]

CASE NO	PRIMARY INFECTION TREATED WITH ANTIBIOTICS	SYMPTOMS OF DISEASE	ISOLATION OF C ALBICANS BEFORE AND AFTER TREATMENT				NYSTATIN ORALLY IN POWDER FORM DAILY DOSE		NO OF DAYS	CLINICAL COURSE OF MONILIASIS
			MOUTH	STOOL	URINE	BLOOD	MG	UNITS		
14	MENINGITIS	THRUSH DYSPHAGIA	+ 0	+ 0	0 0		1,000 500	1,400 000 700,000	1 3	RECOVERY IN 3 DAYS
15	BRONCHOPNEUMONIA	THRUSH DIGESTIVE TROUBLES WEIGHT LOSS	+ 0 + 0	+ 0 + 0	0 0 0 0	0	1 000 500	1,400,000 700,000	4 4	RECOVERY IN 2 DAYS RELAPSE FOLLOWED BY RECOVERY
16	STAPHYLOCOCCOSIS	THRUSH TOXEMIA DIGESTIVE TROUBLES	+ 0 0	+ + 0	0 0 0	0	1,000	1,400 000	2	RAPID RECOVERY
17	SEVERE ANEMIA	THRUSH	+ 0	+ 0	0 0		600	840 000	4	RAPID RECOVERY
18	TOXICOSIS	THRUSH WEIGHT LOSS DIARRHEA	+ 0	+ 0			250	500,000	4	RECOVERY IN 24 HOURS WEIGHT REGAINED IN 4 DAYS
20	RESPIRATORY INFECTION	THRUSH DIARRHEA ++	+ 0	+ 0	0		500	700,000	4	RAPID RECOVERY
23	PREMATURE (NOT TREATED WITH ANTIBIOTICS)	THRUSH	+ 0	+ 0			150	300,000	4	RAPID RECOVERY RELAPSE AFTER 15 DAYS

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FRANCO FLARER

First Results in Italy with a New Antimycotic, Nystatin

Hazen and Brown in 1950 discovered a species of Streptomyces which produced substances possessing broad antimycotic activity Progress has been rapid toward the isolation and standardization of one of these substances namely nystatin so that at present material with a potency of 400 units per milligram is available for clinical use

This abstract summarizes our data in respect to the use of an ointment with a carbowax glycol propylene base and containing 5000 units of nystatin per gram Twelve patients with various dermatophytoses were treated 8 cases of dermatophytosis of the feet 2 cases of trichophytosis and 2 cases of inguinal epidermophytosis

The average period of treatment was twelve days There were no signs of intolerance to the drug even in those patients who had considerable eczematization associated with their dermatophytosis of the feet

Good clinical results were obtained in the cases of dermatophytosis of the feet In 4 cases recovery was considered complete in the remaining 4 insufficient time has elapsed for a final evaluation

The results against the specific fungus infections (trichophytosis and epidermophytosis) were rapid and favorable In addition to the local application of nystatin ointment in the patients with trichophytosis the diseased hairs were epilated and the patients were given orally four tablets of nystatin per day (500 000 units per tablet)

Although only a small number of patients have been treated we can tentatively say that nystatin appears to be well tolerated and to have clinical value in the treatment of dermatophytoses

JAMES H GRAHAM, EDWIN T WRIGHT,
VICTOR D NEWCOMER,
and THOMAS H STERNBERG

The Use of Nystatin As a Topical Antifungal Agent

Nystatin * an antibiotic isolated from *Streptomyces noursei* by Hazen and Brown [1-] has been shown to possess in vitro activity against both saprophytic and pathogenic fungi including *Candida albicans* [1,4] In vivo activity has been demonstrated against *C albicans*, *Histoplasma capsulatum*, and *Coccidioides immitis* [5,8] Several clinical reports have appeared regarding the parenteral and topical use of nystatin in man [9,13] These studies have demonstrated the effectiveness of nystatin against *C albicans* In general it has been shown to be relatively nontoxic and well tolerated This paper deals with the topical use of nystatin in the treatment of 187 patients with various superficial fungus infections

MATERIALS AND METHODS

The majority of these patients were adult males whose ages varied from twenty two to seventy nine years A number of women and children were also included The type of fungus infection was determined by the clinical appearance of the lesions and confirmed by the presence of a positive culture on Sabouraud s media In those infections due to *C albicans* periodic subcultures were made on cornmeal agar to demonstrate the presence of chlamydospores

Nystatin was applied topically in several different forms These included ointments solutions powders troches capsules vaginal suppositories and vaginal gel

* The nystatin used in this study was supplied through the courtesy of the Squibb Institute for Medical Research

The ointment and gel consisted of a plasticized petrolatum base * containing 5000 to 100 000 units of nystatin per gram A second ointment was used containing 200 000 units of nystatin 2 5 mg of neomycin and 0 25 mg of gramicidin per gram of ointment base Two types of solutions were used containing 5000 to 100 000 units of nystatin per cubic centimeter One was a solution of propylene glycol and the other was a solution containing 2 per cent procaine and 0 25 per cent Tween 80 with and without 2 5 mg of hydrocortisone The powder contained 175 000 units of nystatin per teaspoon The troches contained 2000 units of nystatin each In addition some contained 2 5 mg of neomycin and 0 25 mg of gramicidin The capsules and tablets which were used as troches and suppositories contained 125 000 to 500 000 units of nystatin The vaginal suppositories contained 10 000 units of nystatin and in addition some contained 2 5 mg of neomycin and 0 25 mg of gramicidin

Cutaneous infections were treated with ointments and solutions three times daily Oral infections were treated with solutions troches and capsules and tablets used as troches four times daily In addition the powder was used orally four times daily and administered by mixing one teaspoonful in a glass of milk or water Infections of the female genitalia were treated once daily with the use of disposable vaginal applicators containing the gel vaginal suppositories and tablets and capsules used as suppositories

Treatment periods varied from three days to eight months The results are summarized in Table 1

DISCUSSION

The results of treatment were judged on both the clinical response and laboratory demonstration of the fungus The clinical response was graded as no change fair good and excellent Laboratory follow up was determined by performing potassium hydroxide mounts and/or demonstrating the fungus by culturing it on Sabouraud s media

A total of 94 patients were treated with infections due to *C albicans* These included 30 patients with oral moniliasis 15 with vaginal moniliasis and 49 with cutaneous involvement Treatment

* Plastibase Squibb

TABLE 1

RESULTS OF TREATMENT WITH TOPICAL NYSTATIN

I. ORAL MONILIASIS

Form of Drug	Dosage	Route	Period	No of Patients	Results	REMARKS
Troches	1 qid	oral	3 - 42 days	14	Excellent -4 Good -10	All patients were clinically clear, in 5, repeat cultures were positive at the end of the treatment period
Tablets	1 qid	oral	3 - 14 days	8	Excellent -6 Good -1 Fair -1	Seven of the 8 patients were clinically clear, in 3, repeat cultures were positive at the end of the treatment period
Troches	1 qid	oral	3 - 8 mos	3	Good	All patients were clinically controlled; C albicans was repeatedly cultured during and following treatment period
Tablets	"	"				
Powder	1 teasp qid	"				
Tablets	1 qid	oral	21-48 days	2	Excellent	Patients were clinically clear and C albicans could not be cultured after treatment
Solution	qid	"				
Capsules	1 qid	oral	3 mos	2	Good	Both patients were well controlled, lesions of C albicans reappeared one week after treatment was discontinued Cultures remained positive during and after treatment
Solution	qid	oral	7 days	1	Excellent	Clinically clear and the culture was negative at the end of treatment

II VAGINAL MONILIASIS

Form of Drug	Dosage	Route	Period	No of Patients	Results	Remarks
Gel	qid	intra-vaginal	7 days	7	Excellent	
Tablets	1 qid	intra-vaginal	3 da to 3 mos	3	Good	All 15 patients were clinically clear; in 6, C albicans was recovered from cultures at the end of the treatment period
Capsules	1 qid	intra-vaginal	3 to 7 days	2	Good	
Suppositories	1 qid	intra-vaginal	7 to 10 days	2	Excellent	
Tablets Ointment Suppositories	1 qid qid 1 qid	intra-vaginal	6 wks	1	Good	

III CUTANEOUS MONILIASIS

Form of Drug	Dosage	Route	Period	No of Patients	Results	Remarks
Ointment	tid	topically	1-12 wks	22	Excellent -10 Good -10 Fair - 2	20 of the 22 patients were clinically clear From 7 of the 20, C albicans was recovered from cultures after the treatment period
Solution	tid	topically	4-30 days	21	Excellent -10 Good - 9 Fair - 2	19 patients were clinically clear; 13 of the group had positive cultures for C albicans at end of the treatment period
Ointment Solutions	tid "	topically	1-3 wks	6	Good	All patients were clinically clear Cultures were positive for C albicans at the end of the treatment period

TABLE 1

RESULTS OF TREATMENT WITH TOPICAL NYSTATIN

IV CUTANEOUS MYCOSES

Form of Drug	Dosage	Route	Period	No of Patients	Results	Remarks
Ointments Solutions	tid "	Topical	1 wk to 8 mos	60	Excellent -1 Good -3 Fair -28 No Change -28	T rubrum: All patients except one had positive cultures at end of the treatment period The majority of the patients were treated for a minimum of 3 months
			2 wks to 3 mos	26	Excellent -1 Fair -20 No change -5	T gypseum: All patients except one had positive cultures at end of the treatment period The majority of the patients were treated for a minimum of 3 months
			2 wks to 3 mos	3	Excellent -1 Good -2	M lanosum: Cultures were negative at the end of the treatment period
			16 days	1	Excellent	M fulvum: Cultures were negative at the end of the treatment period
			14 days	1	Good	M furfur: At the end of the treatment period the fungus was demonstrated by potassium hydroxide mount
			30 days	1	Good	E floccosum: Cultures were positive at the end of the treatment period
			3 mos	1	No change	T tonsurans: Cultures were positive at the end of the treatment period

periods varied from three days to eight months Clinical response was in general good to excellent This was often dramatic in cases of thrush and perlèche the lesions frequently disappearing within forty-eight to seventy two hours However in general clinical clearing occurred within two to three weeks Relapses were infrequent and when they did occur were noted within one week after discontinuance of the drug No resistance to the drug was noted in patients being re treated or treated for prolonged periods of time Relapses were seen most frequently in patients suffering from serious diseases such as pemphigus vulgaris systemic lupus erythematosus leukemia and severe diabetes

All the preparations of nystatin used were effective in the various concentrations used When hydrocortisone was added to the solutions erythema decreased rapidly and in many patients subjective sensations of itching and burning subsided within twenty four hours The presence of neomycin and gramicidin provided no additional clinical benefit The majority of patients were seen two to three times weekly Potassium hydroxide mounts and/or cultures on Sabouraud s media were done after clinical clearing of the lesions occurred It was noted that in a number of patients with severe systemic illnesses and particularly diabetes there was no clinical evidence of moniliasis at the end of the treatment period however *C albicans* could still be demonstrated by culture

There were 93 patients with dermatophytosis including 60 with *Trichophyton rubrum* 26 with *T gypseum* 3 with *Microsporum lanosum* and 1 each with *M fulvum M furfur Epidermophyton floccosum* and *T tonsurans* infections The results of treatment in this group were in general less dramatic than in those patients with moniliasis Treatment periods varied from one week to eight months the average being three months Clinical improvement was much slower There was little difference in the response to the treatment of the various dermatophyte infections In the majority of instances even though there was clinical improvement the cultures were still positive at the end of the treatment period Clinical results were better when preparations containing higher concentrations of nystatin were used In those patients treated with preparations containing hydrocortisone and nystatin erythema and pruritus when present subsided in twenty four to forty eight hours Here again clinical improvement was good but repeat cul

tures were positive This was especially true of dermatophyte infections due to *T rubrum*

All the preparations of nystatin were acceptable for patient use There were no instances of allergic contact dermatitis or of primary irritation

It is our impression from this study that nystatin gives good to excellent results when used for the treatment of moniliasis It is less effective when used for the treatment of superficial dermatophyte infections

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ANTONIO GONZÁLEZ OCHOA

Two Cases of Abdominal Wall Moniliasis, after Colostomy, Treated with Nystatin

The much discussed increase in intercurrent mycotic infections resulting from the widespread use of the broad spectrum antibiotics has been exaggerated by some authors and de emphasized or denied by others Nevertheless one fact concerning moniliasis remains incontrovertible This is the significant overgrowth of *Candida albicans* in the intestinal and respiratory tracts following prolonged administration of the broad spectrum antibiotics From this fact it is possible to draw the inference that the increasing number of subjects with large numbers of *C albicans* suggests that some cells of this yeastlike population acquire pathogenicity and produce disease

Even though systemic moniliasis is not common everyone who works in clinical mycology has observed authentic cases and the clinicians must be acquainted with this disease When *C albicans* is isolated from closed abscesses and body fluids such as blood and ascitic fluid the mere isolation makes the diagnosis since the microorganism does not exist there as a normal inhabitant However in other pathologic substrata such as open abscesses ulcers sputum or stools the diagnosis of moniliasis must be made with considerable caution The reasons for this are well known

The usual agents employed in the treatment of systemic moniliasis such as gentian violet potassium iodide and ethyl iodide have little or no effect and the cures reported with such medicaments make the initial diagnosis doubtful

The marked activity of nystatin against some pathogenic fungi particularly its capacity to suppress Candida has been shown by many investigators from the experimental point of view [1,2] These studies have been followed by clinical investigations concerning

the treatment of intestinal [5] oral [6-12] vaginal [13-16] cutaneous [17-22] and systemic moniliasis [23-27]

Chronic regional ileitis or regional enteritis is well known to surgeons for its almost hopeless prognosis in elderly patients The operative therapy consists of resection of the involved ileum and colon but the high recurrence rate after resection indicates that surgery alone has not proved adequate and additional measures are absolutely necessary Sulfonamides and antibiotics to date have had no demonstrable effect upon the primary inflammatory process and no particular benefit has resulted from their long continued administration

This paper deals with two cases of regional enteritis which presented a marked similarity in clinical picture including therapy with broad spectrum antibiotics before surgical treatment and a severe postoperative course in spite of antibiotic therapy Each patient had ulceration of the abdominal wall around the colostomy sutures Large numbers of *C albicans* were found in the exudates as well as in the stools The oral and local administration of nystatin in both cases was accompanied by a noteworthy improvement of the ulcers defervescence and a favorable change in the general condition and eventual recovery

REPORT OF CASES

The first patient a 65 year-old man complained of indefinite attacks of pain in his right lower abdomen accompanied at times by vomiting low fever and moderate leukocytosis (about 10 000) Six courses of broad spectrum antibiotics were administered over a period of eight months Tetracycline and chloramphenicol were given in an average dose of 1 gm daily The last and most severe attack persisted for three days with a clinical picture of high fever vomiting and a leukocytosis of 18 000 A clinical diagnosis of ileocecal intussusception was entertained and a laparotomy was deemed necessary A segmental phlegmon was found involving the coats of the terminal ileum and cecum An anastomosis between the ileum and transverse colon was made and a colostomy performed The patient s condition remained critical with prostration and fever despite administration of antibiotics Two days later the skin around the colostomy sutures began to ulcerate ac

companied by a profuse sanguinopurulent discharge Microscopic examination revealed numerous yeastlike cells and short pseudomycelial elements diagnostic of *C albicans* Large numbers of this organism were also seen in the stools

The patient s condition continued to deteriorate and eleven days later a course of nystatin was initiated which consisted of the daily administration of 1 5 million units orally Nystatin in suspension was also introduced into the lumen of the colostomy, and nystatin powder was applied to the ulcers Two days later a marked improvement was observed which paralleled the disappearance of *C albicans* On the sixth day of treatment with nystatin the fever subsided and the ulcers began to heal By the ninth day the patient had completely recovered and treatment with nystatin was discontinued

The second patient a 67 year old woman had had periods of tenderness in the right abdominal region for seven months prior to admission She was hospitalized because of an acute attack with intense pain fever and leukocytosis and had received courses of broad spectrum antibiotics during her earlier attacks Physical examination revealed a mass localized in the right lower quadrant and a laparotomy disclosed a mass involving both the cecum and terminal ileum An anastomosis was made between the ileum and transverse colon and a colostomy was performed No malignancy was observed on microscopic examination of histologic sections of the removed tissues The postoperative course was similar to that in the first case in that this patient showed continued fever toxemic signs diarrhea and phlegmonous ulceration in the abdominal wall around the colostomy These manifestations did not subside with the administration of antibiotics Large numbers of *C albicans* were seen in the exudate from the ulcers and in the stools Two weeks after surgical intervention nystatin was started both locally and orally as in the first case The same favorable results were obtained with defervescence healing of the ulcerations and remarkable systemic improvement within nine days During this period suppression of *C albicans* was also observed No side effects attributable to the use of nystatin were observed in either patient

DISCUSSION

As we have emphasized these two patients strikingly resembled one another in the clinical chronicity of their disease in the lesions found at laparotomy in the postoperative course and in the results obtained with nystatin During their chronic course both patients received broad spectrum antibiotics on numerous occasions

It is difficult to say to what extent the presence of *C albicans* was responsible for the severe preoperative and postoperative course However the patients did recover and *C albicans* did dis appear from their abdominal ulcers and stools when nystatin was given This suggests that it will be worth while to make similar trials in the future

Finally the etiology of chronic terminal ileitis is doubtful Bac terial protozoal and viral agents have been considered but the current opinion is that none of these has a primary etiologic rela tionship to the disease Since most of these patients receive numer ous courses of sulfonamides and broad spectrum antibiotics during the chronic stages of the disease we should consider the possibility that *C albicans* plays a part in the etiology of this ailment or is a complicating agent which should not be overlooked in future in vestigations

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17 Sloane To be published
18 Bereston To be published
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20 Dillaha To be published
21 Newcomer To be published
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27 Drouhet To be published

F MARIAT

The Action of Nystatin* on the Growth of *Spor trichum schenckii* and on Its Behavior in Vivo

The antifungal antibiotic nystatin discovered by Hazen and Brown [1] has been evaluated in vitro and in vivo against pathogenic fungi and they [2] have listed the fungi which are inhibited by this new antibiotic Other papers have appeared concerning the antifungal action of nystatin in vitro or in vivo against *Candida albicans* *Histoplasma capsulatum* and *Coccidioides immitis* [3-6] Drouhet [7,8] obtained interesting results with *C albicans* both experimentally and in the treatment of humans Segretain [9] has observed an inhibition of growth of many strains of fungi isolated from Madura foot

The effect of nystatin on *Sporotrichum schenckii* the fungus which causes sporotrichosis was first studied by Hazen and Brown [2] Growth was inhibited in vitro on solid medium in a concentration of 13 μg /ml Campbell et al studied the action of nystatin on experimental sporotrichosis in mice

The first results of our work concerning the effect of nystatin on *Sporotrichum schenckii* in vitro and in vivo are reported here

MATERIALS AND METHODS

IN VITRO EXPERIMENTS

All experiments were performed employing the following liquid medium KH_2PO_4 0 36 gm $Na_2HPO_4 \cdot 12H_2O$ 1 42 gm $MgSO_4 \cdot 7H_2O$ 0 6 gm KCl 1 00 gm casein hydrolysate 10 gm solution

* The nystatin used in this study was kindly supplied by Squibb & Sons New Brunswick New Jersey

of micronutrients, 10 drops glucose 10 gm bidistillated water 1000 ml pyrimidin of thiamine 10^{-6} biotin 10^{-9} (pH 6 9) This solution was distributed in Legroux tubes and in Erlenmeyer flasks The antibiotic (lots HL–162–G HL–99–E and St 695–21/15 F) was added to the medium to make a final scale of concentrations ranging from 24 to 4 μg /ml All these concentrations were made from an initial solution of nystatin (480 μg /ml) in 70 per cent alcohol buffered at pH 7 The same quantity of alcohol (3 5 per cent) as contained by the medium with the highest concentration of nystatin was added to controls (such a percentage of alcohol in media does not prevent growth of fungi) Penicillin (25 μg /ml) and streptomycin (50 μg /ml) were also added to the media to prevent the development of bacteria present in the nystatin powder which were not killed by alcohol Penicillin and streptomycin do not act in any way on the growth of *S schenckii* in vitro or on experimental sporotrichosis [10]

The inoculum consisted of a drop of a suspension of a conidia or of yeast cells of the fungi Two strains were used a white one *S schenckii* (IP n°29) and a black one *S schenckii beurmanni* (IP n°30) The evaluation of growth of the fungi was made by dry weights The mycelial phase was prepared from cultures grown for fourteen days at 30°C and the yeastlike phase was prepared from cultures grown for ten days with shaking at 37°C [11, 12] Three to seven flasks were used for each concentration

IN VIVO EXPERIMENTS

Male hamsters were used The animals were inoculated intraperitoneally with a suspension of the yeast phase of *S schenckii* (about 5 million living cells) Nystatin 25 μg /ml of penicillin and 50μg /ml of streptomycin were injected intraperitoneally in different concentrations and at different times

RESULTS

IN VITRO EXPERIMENTS

Mycelial Phase In a first series of experiments *S schenckii* was inoculated into media containing 50 25 12 5 6 25 3 1 25 0 μg /ml of nystatin After culturing for fourteen days at 30°C

the dry weights of the mycelium were obtained A significant inhi bition occurred in the media containing 6 25 µg /ml of nystatin No growth of the fungus occurred in those media containing 50 and 25 µg /ml

Experiments summarized in Table 1 are performed with a more accurate scale of concentrations The antifungal action of nystatin is significant between concentrations of 8 and 12 µg /ml

TABLE 1

GROWTH OF *SPOROTRICHUM SCHENCKII* AND *S SCHENCKII BEURMANNI* (MYCELIAL PHASE) IN DIFFERENT CONCENTRATIONS OF NYSTATIN

Concentrations of Nystatin (µg /ml)	*Dry Weights of Mycelium (mg /ml of medium)*	
	S schenckii	*S schenckii beurmanni*
24	0 10	0 45
20	0 20	0 95
16	1 00	0 85
12	4 55	1 55
8	6 45	2 11
4	5 60	2 45
0	5 50	2 50

Yeast Phase In the yeast phase *S schenckii* grows more rapidly than in its mycelial phase No growth of *S schenckii* was observed with 12 µg /ml of nystatin in the first experiment The experiment was repeated several times but the same sensitivity was never ob served The results obtained were always similar to those shown in Table 2 where inhibition was noted in concentrations of 8–16 µg /ml

Fungicidal Action Suspensions of the yeast phase of *S schenckii* were mixed with two solutions of nystatin (200 and 400 µg /ml) and shaken for eighty two hours At time zero the mixtures con tained about 2 000 000 living cells per milliliter After fourteen hours and after eighty two hours no living cells were recovered upon culturing Sporotrichosis did not develop in hamsters inocu lated with such suspensions

In another experiment a contact of twenty six hours between *S schenckii* and a solution of nystatin (100 µg /ml) was not suf ficient to kill all the cells of the fungus These same washed cells

TABLE 2

GROWTH OF *SPOROTRICHUM SCHENCKII* AND *S SCHENCKII BEURMANNI* (YEAST PHASE) WITH DIFFERENT CONCENTRATIONS OF NYSTATIN

Concentrations of Nystatin (µg /ml)	*Dry Weights of Fungi (mg /ml of medium)*	
	S schenckii	*S schenckii beurmanni*
24	0 25	0 0
20	0 05	0 40
16	2 35	1 45
12	3 50	1 55
8	4 00	2 80
4	4 70	4 10
0	4 30	4 00

when subsequently inoculated into hamsters produced sporotrichosis

IN VIVO EXPERIMENTS

Generally within four to eight days after inoculation with *S schenckii* all male hamsters develop an orchitis which rapidly breaks down and drains Characteristic lesions are also observed on the paws The animals die after a variable length of time not only of the sporotrichosis (*S schenckii* is found in the viscera) but also of secondary infections [18]

Sporotrichosis does not develop when the fungus and a solution of nystatin are introduced into the peritoneal cavity at the same time Thirteen male hamsters were inoculated with the fungus and 450 µg /ml of nystatin After four and one half months 12 of the 13 animals showed some evidence of sporotrichosis The thirteenth as did the controls developed a severe orchitis and died after two and one half months

We are now in the process of studying the therapeutic effect of nystatin when initiated at various time intervals following inoculations of *S schenckii* into hamsters and guinea pigs The results will be published elsewhere For the present it can be said that in the case of hamsters delayed treatment with nystatin (1000 µg the fifth day after inoculation with *S schenckii*, 500 µg the sixth seventh eighth and ninth days 2000 µg the twelfth day 1000 µg

the thirteenth fourteenth fifteenth and sixteenth days) does not prevent the development of sporotrichosis

CONCLUSIONS AND SUMMARY

Like other pathogenic fungi studied *S schenckii* and its variety *S schenckii beurmanni* were found to be sensitive to the antifungal action of nystatin In vitro the growth of the yeast phase (the parasitic phase) as well as that of the mycelial phase (the saprophytic phase) are inhibited by concentrations of the product ranging from 8 to 16 μg /ml There is practically no growth at concentrations of 20 and 25 μg /ml It appears that after a period of time such as that required for the incubation of Sporotrichum nystatin loses part of its activity The threshold for nystatin activity must be established with adequate precision

Modifications of the morphology of the fungi were never found in our experiments

The development of *S schenckii* is also inhibited in vivo but it seems that the action of nystatin is impeded by the fixation of the fungus in the tissues However Campbell and others[6] obtained an increased percentage of survival in mice with experimental sporotrichosis when treatment was delayed (three days) The dosages of nystatin used in their experiments were much higher than ours

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EDOUARD DROUHET and JAN SCHWARZ

Evaluation of the Action of Nystatin (Mycostatin) on *Histoplasma capsulatum* in Vitro and in Hamsters and Mice*

Hazen and Brown[1] demonstrated the inhibitory action of nystatin on *Histoplasma capsulatum* in the yeast phase with a concentration of 1 to 6 μg per milliliter of media

They also demonstrated prolongation of survival rate in mice after intraperitoneal inoculation of the yeast phase of *H capsulatum* when nystatin was given simultaneously These results were essentially confirmed by Campbell et al[2] in a variety of schedules of treatment of mice They were able to show that the prolongation of the survival rate was greatly dependent on the time of initiation of treatment

The nystatin used in the following experiments was lot HL-185 †

One milligram of the lot was equivalent to 2500 units Fourteen strains were tested in the mycelial phase 8 in the yeast phase The former were grown on Sabouraud s glucose agar at room temperature the latter on cystein blood agar at 37°C

Of the 14 strains in the mycelial phase all were inhibited with as little at 2 5 units in the first three to five days while most controls developed quite visible colonies At seven days 13 of the 14 tested strains showed inhibition with 5 units and only one was growing At ten days 7 showed inhibition with 10 units and at twenty five days 6 showed inhibition with 20 units At this time only 3 strains were growing in the presence of 50 units per milli

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† The material used in this work was supplied through the courtesy of Harvey Blank M D., Squibb Institute for Medical Research

TABLE 1

FUNGISTATIC ACTIVITY OF NYSTATIN IN VITRO ON MYCELIAL PHASE OF 14 DIFFERENT STRAINS OF HISTOPLASMA

The figures refer to size of colony in millimeter of diameter Readings were taken daily but only eight readings are reproduced

	µg of Nystatin/cc of medium								µg of Nystatin/cc of medium								µg of Nystatin/cc of medium							
	0	2.5	5	10	15	20	30	50	0	2.5	5	10	15	20	30	50	0	2.5	5	10	15	20	30	50
Days of Cul	strain 18								strain 19								strain 20							
3	3	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
7	6	4	0	0	0	0	0	0	8	3	0	0	0	0	0	0	10	2	0	0	0	0	0	0
10	12	7	3	0	0	0	0	0	13	6	0	0	0	0	0	0	14	5	0	0	0	0	0	0
13	16	10	6	0	0	0	0	0	18	9	0	0	0	0	0	0	18	8	0	0	0	0	0	0
16	23	16	10	4	0	0	0	0	25	14	0	0	0	0	0	0	23	14	4	2	0	0	0	0
19	25	20	13	8	4	0	0	0	28	19	4	4	0	0	0	0	26	17	7	6	0	0	0	0
22	27	22	17	11	8	9	0	0	30	21	6	6	2	0	0	0	30	21	10	8	0	0	0	0
25	30	28	20	13	11	0	0	0	32	26	10	10	6	0	0	0	31	24	12	8	6	0	0	0
Days of Cul	strain 27								strain 31								strain 35							
3	2	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
7	8	4	0	0	0	0	0	0	7	4	3	2	0	0	0	0	8	4	4	4	3	0	0	0
10	12	6	4	0	0	0	0	0	13	9	8	6	3	0	0	0	11	10	8	6	5	3	0	0
13	16	8	7	3	0	0	0	0	21	16	13	12	4	0	0	0	16	13	10	9	7	5	3	0
16	18	14	10	3	2	0	0	0	30	22	20	17	10	0	0	0	20	17	12	13	12	7	4	0
19	20	16	12	4	4	0	0	0	32	28	26	23	16	0	0	0	25	20	19	16	14	9	7	0
22	22	18	14	8	6	0	0	0	35	32	28	27	18	0	0	0	26	22	20	18	15	10	7	0
25	25	22	20	10	8	0	0	0	38	35	30	30	24	0	0	0	27	25	24	18	16	11	7	0
Days of Cul	strain 44								strain 63								strain 65							
3	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
7	3	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0
10	8	5	8	4	2	2	2	0	2	2	2	2	2	0	0	0	11	0	0	0	0	0	0	0
13	10	9	10	6	6	6	6	3	6	3	3	3	3	0	0	0	16	2	2	2	0	0	0	0
16	15	11	15	10	8	8	6	6	10	10	10	8	8	2	2	0	22	4	4	3	2	0	0	0
19	19	14	19	14	13	13	12	11	15	14	14	11	11	5	4	3	27	6	6	5	4	2	2	0
22	22	15	22	15	15	15	14	14	18	17	17	16	16	9	8	6	29	9	8	7	6	4	4	0
25	26	18	26	17	17	17	16	16	20	19	19	18	11	11	11	8	31	13	10	8	8	6	6	0

Days of cult.	strain 6522								strain 6625								strain F.D.T.P.							
3	2	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	4	3	3	2	0	0	0	0	3	2	0	0	0	0	0	0	3	0	0	0	0	0	0	0
10	8	6	6	5	0	0	0	0	7	4	0	0	0	0	0	0	5	3	2	0	0	0	0	0
13	13	12	11	8	4	0	0	0	10	7	3	3	3	2	0	0	7	5		0	0	0	0	0
16	18	16	15	14	8	0	0	0	1	12	6	6	6	5	4	0	11	6	6	3	3	2	0	0
19	22	21	20	19	13	4	2	0	18	16	9	9	9	8	7	0	16	9	7	5	5	5	0	0
22	24	24	22	17	19	9	7	3	22	20	14	14	13	13	13	0	20	12	10	8	8	8	5	0
25	28	26	23	22	20	11	9	5	25	23	17	17	15	15	15	0	23	15	15	10	10	10	7	0

Days of cult.	strain 98 T.P.								strain 261 T.P.							
3	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
7	3	0	0	0	0	0	0	0	6	3	2	0	0	0	0	0
10	4	0	0	0	0	0	0	0	13	6	6	3	2	0	0	0
13	5	2	2	2	0	0	0	0	17	11	9	4	2	0	0	0
16	6	5	3	4	0	0	0	0	22	16	14	8	6	4	0	0
19	8	6	4	5	3	0	0	0	26	20	19	12	8	7	0	0
22	9	7	5	5	4	0	0	0	27	26	25	18	12	11	0	0
25	20	9	7	7	6	0	0	0	30	30	28	20	14	14	4	0

SENSITIVITY TO NYSTATIN

OF VARIOUS STRAINS OF

HISTOPLASMA.

CULTURE ON SABOURAUD MEDIA AT 25 C

INOCULUM, A FRAGMENT OF MYCELIUM

liter but the size of the colonies was markedly smaller than in the controls (Table 1)

Of the 8 strains in the yeast phase inhibition was obtained with 10 units in 1 strain for three days in 2 for ten days Twenty units inhibited 6 strains for about ten days and fifty units inhibited all strains except one which was also considerably resistant in the mycelial phase (Table 2)

From this we can conclude that the yeast phase seems to be less sensitive to the action of nystatin than the mycelial phase But one must take into consideration that nystatin is destroyed more rapidly at incubator temperature and that the media used for the yeast phase are much more complex and interference with the fungistatic activities may well occur

TABLE 2

SENSITIVITY OF HISTOPLASMA TO NYSTATIN ON BLOOD-CYSTEIN-AGAR MEDIUM AT 37 C (YEAST PHASE)

	STRAINS	DAYS	NYSTATIN UNITS/ml of media 0	2 5	5	10	15	20	30	50
AMERICAN STRAINS	5	3		+		0	0	0	0	0
		7	++	++	++	+	±	0	0	0
		12		+	++	+++	+	±	0	0
		15		+				+	0	0
	27	3	++					0	0	0
		7						0	0	0
		12	+		+++	++	+	0	0	0
		15	++		++	++	++	0	0	0
	31	3				++	±	0	0	0
		7		++			+	0	0	0
		12			+	++	+	±	0	0
		15	+++	+	++	++	+++	+	0	0
	69	3		+	±	0	0	0	0	0
		7		+	+	0	0	0	0	0
		12		++		-	+	0	0	0
		15		++		++		0	0	0
	2764	3						0	0	0
		7	+++	+	+	+	+ +		0	0
		12	+	++++				++	+	0
		15	+ ++							0
AFRICAN	6621	3			+		+	0	0	0
		7		+	+		++	0	0	0
		12		+				0	0	0
		15				+			0	0
	261 I P	3				++		0	0	0
		7			+++				0	0
		12	+	++	+	+				±
		15	++++	+			+			+
	44	3				0	0	0	0	0
		7				0	0	0	0	0
		12	+					0	0	0
		15							±	

ANIMAL EXPERIMENTS

HAMSTERS

Hamsters were selected because it was shown that this species is much more susceptible to infection with *H capsulatum* than mice In addition they offer an advantage for the experimenter inasmuch as mortalities occur in relatively few days which permits evaluation of treatment with great ease[9] Two separate experiments were made with hamsters the first with a moderately virulent strain the second with a fulminatingly virulent organism

First Experiment One hundred and three male hamsters ap-

proximately 25 to 30 gm in weight were injected intraperitoneally with 35 million yeast cells of our strain 19 isolated from a patient in Cincinnati with disseminated lethal histoplasmosis The strain was grown on Kurung s [5] media for about ten days The following results were obtained After approximately eight weeks the mortality of the controls was 87 per cent The animals treated simultaneously with the inoculation had a mortality of 10 per cent Animals treated seven days after inoculation had a mortality of 60 per cent But 6 of the 9 animals died within five days after initiation of treatment and liberation of toxin from destroyed yeast cells must be considered Control animals treated exclusively with nystatin showed no toxic effects

Cultures from all animals were positive except those from 4 hamsters of the group which was treated with 1250 units per day simultaneously with inoculation

Microscopic examination of the principal organs revealed as the main difference between treated and control animals the fact that spread to the lungs was practically eliminated and involvement of the liver and spleen markedly decreased in the treated animals The difference in organ involvement was parallel to the amount of nystatin given and to the time of the initiation of treatment

From this experiment it seems obvious that treatment should be started early but with slowly increasing dosage with nystatin which we learned later should be given twice daily (Tables 3 and 4 Fig 1)

Second Experiment In the second experiment with 116 hamsters 80 were injected with 50 million yeast cells grown on Kurung s [8] media of strain 27 isolated from a dog in Cincinnati which died of disseminated histoplasmosis 36 animals were injected with 25 million organisms intraperitoneally

All controls injected with 50 million organisms were dead within sixteen days At this time of the animals injected with nystatin one hour prior to inoculation 15 were still alive Of 20 treated for the first time six hours after inoculation 12 were alive and of 20 treated three days after inoculation 5 were alive Of the control hamsters inoculated with 25 million yeast cells 16 of 18 died within sixteen days and all hamsters were dead in twenty days Before treatment was started microscopic examination of two sacrificed hamsters showed severe and generalized histoplasmosis Treatment

TABLE 3

EFFECT OF NYSTATIN ON HAMSTERS EXPERIMENTALLY INFECTED WITH THE YEAST PHASE OF *HISTOPLASMA CAPSULATUM* (STRAIN 19) BY INTRAPERITONEAL ROUTE 103 HAMSTERS

Group	Number of hamsters	Inoculum in millions of yeast cells	Treatment (subcutaneous) Nystatin Lot HL-185 1 mgr 2500 units			Number of hamsters	
			treatment started	daily dose	number of days	sacrificed	dead after 58 days
HA I	21	35				6	13/15
HA II	10	-		625 U	58	0	0/10
HA III	10	-		1250 U	58	0	0/10
HA IV	20	35	simultan w inocul.	625 U	58	6	1/14
HA V	21	35	simultan w inocul.	1250 U	58	6	2/15
HA VI	21	35	7 days p inocul	625 U 1250 U	46 (lot a) 46 (lot b)		

°Initial body weight of hamsters 25–30 grams

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thus was started in progressively sick animals seven days after inoculation Sixteen days after inoculation 7 out of 18 were still alive but died subsequently within twenty three days

The extremely fulminating course of disease with strain 27 was considerably retarded with all schedules of treatment Again the benefit of treatment depended largely on the time when treatment started and on the amount given (Table 5 Fig 2)

TABLE 5

EFFECT OF NYSTATIN ON HAMSTERS EXPERIMENTALLY INFECTED WITH THE YEAST PHASE OF *HISTOPLASMA CAPSULATUM* (STRAIN 27) BY INTRAPERITONEAL ROUTE 116 HAMSTERS

Group	Number of hamsters	Inoculum in millions of yeast cells	TREATMENT (subcutaneous) Nystatin Lot HL-165 1mgr=2300 Units	Total dose	Number of dead hamsters after 16 days
B I	20	50			20/20
B II	20	50	1150 U 1 hour before inocul 1150 U 2 hours after inocul 2nd-6th days 1150 U in AM 2300 U in PM after 3 days rest same schedule for 5 days	16 mg* 36 300 U given in 11 days	5/20
B III	20	50	1150 U 6 hours after inocul subsequently identical schedule as B II	15 5 mg 35.650 U given in 11 days	8/20
B IV	20	50	Treatment started 3 days after inoc 2 days 287 U AM + 287 U PM 2 days 575 U AM + 575 U PM 4 days 1150 U AM + 1150 U PM 4 days 1150 U AM 2300 U PM	11 5 mg 26.400 U given in 12 days	15/20
B V	18	25			16/18
B VI	18	25	Treatment started 7 days after inoc 1 day 287 U AM + 287 U PM 1 day 575 U AM 575 U PM 2 days 1150 U AM + 1150 U PM 4 days 1150 U AM 1150 U PM	8 75 mg 20 125 U 8 days	7/18

* Initial body weight of hamsters 25-30 grams

TABLE 4

PRESENCE OF HISTOPLASMA ORGANISMS ON CULTURE AND MICROSCOPICALLY IN TREATED AND CONTROL ANIMALS (HAMSTERS) GRIDLEY STAIN

Group	Culture		MICROSCOPIC					
			Liver		Spleen		Lung	
	+	−	+	−	+	−	+	−
I	21	0	18	3	20	−*	18	1•
V	17	4	8	13	13	7*	4	17
IV	20	0	16	3°	17	2	8	11
VI	20	1	17	4	19	1*	9	9**

°One case missing *One sple n missing

•Two lungs missing **Three lungs missing

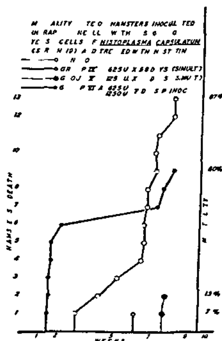


FIGURE 1

Microscopic examination did not reveal striking differences be tween the treated and control animals We were however forced to the conclusion that the many organisms visualized in the tissues which could not be cultured represented dead cells Nine of the treated animals showed such behavior as contrasted with only 2 of the controls

TABLE 6

EFFECT OF TREATMENT WITH NYSTATIN IN EXPERIMENTAL (CHRONIC) HISTO-PLASMOSIS IN MICE

	TR.ATED AI ILALS(28)	CC TROLS(27)
Cultures positive	11	19
Cultur s negative	16	2
Cultures missing or cont minat	1	6
Organisms pr sent microscop	19	14
absent	9	10
slides missing		3
Org isms present in tissu but sterile cult	9	2

Organisms morphologically consistent with *H capsulatum* have been found in the past in human organs and cultures were nega tive in 40 such cases indicating that these organisms remain visible in organs for a long time [*]

In this series of mice with chronic histoplasmosis positive cul tures were obtained in 90 per cent of the controls but in only 40 per cent of the treated animals (Table 6)

Second Series of Mice Close to a hundred animals were treated one hundred days after intraperitoneal inoculation of the yeast phase of strain 18 of which 40 million cells were given This strain was isolated from a fulminating lethal human histoplasmosis

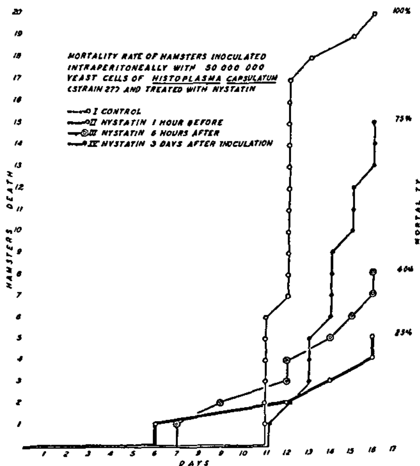


FIGURE 2

MICE

Fifty five animals were injected with the mycelial phase of strain 27 and developed chronic histoplasmosis demonstrated by culture and microscopy Treatment was started four months after inoculation Progressive doses were given once daily starting with 312 units for three days 625 for four and 1250 units for seventeen consecutive days Three days after the cessation of treatment all mice were sacrificed Of 27 controls positive cultures were obtained in 19 negative cultures in 2 and 6 were missing or contaminated Of the 28 treated animals 11 had positive and 16 negative cultures with one missing

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The mortality in both groups was minimal (8 per cent) Treatment was progressive starting with 312 units for three days 625 units for three days and increasing to 1875 units for four days Subsequently after three days rest two courses of 1250 units were given twice daily for four days each All mice were sacrificed after a total length of treatment of twenty eight days this was done two days after treatment was stopped to avoid inhibition of cultural growth by nystatin accumulated in tissue

Definite and marked inhibition of growth was observed in the treated animals but 100 per cent positive cultures were obtained from controls

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LEE E GORDON and CHARLES E SMITH

Mycostatin and Aminostilbamidine Treatment of Experimental Coccidioidomycosis*

The proximity of the San Joaquin Valley and its associated valley fever has provided a great impetus for many investigators in the southwestern United States to maintain a vigilance for therapeutic agents that might be used in the management of coccidioidomycosis

The recently available antibiotics have been tried and found not to be helpful except in cases of concurrent secondary infections Other drugs have likewise been shown to be ineffective or inconclusive These failures may in part reflect the limitations in quantities of drug that can be administered and the fact that their therapeutic levels in the body are not attained Ethyl vanillate can be cited as an example

Our attention was directed to fungicidin an antibiotic reported by Hazen and Brown to be active against *Coccidioides immitis*[1] This antibiotic is now prepared by F R Squibb & Sons under the name of Mycostatin

Another compound which has occupied our interest is aminostilbamidine a stilbamidine derivative Stilbamidine has been used successfully in the treatment of blastomycosis

The present report will deal with our studies on the effect of Mycostatin and aminostilbamidine on experimental coccidioidomycosis

* These investigations were conducted in part under the sponsorship of the Commission on Acute Respiratory Diseases Armed Forces Epidemiological Board in part by the Office of the Surgeon General Department of the Army and in part by a contract between the University of California and the Office of Naval Research

The opinions contained in this report are not to be construed as reflecting the views of the Navy Department or the Naval Service at large (Article 1 5 U S Naval Regulations 1948)

IN VITRO STUDIES

Mycostatin suspended in sterile buffered physiologic saline solution pH 7 4 was added to asparagine medium so that the final concentrations were 0 1 to 1000 μ per milliliter of medium One drop of a saline suspension of *C immitis* was added to each tube and incubated at 35°C It was found that growth of the fungus was suppressed in concentrations of 100 μ per milliliter for thirty days of observation Smaller amounts of antibiotic prevented growth for a few days but with continued incubation growth occurred in these tubes

Aminostilbamidine was prepared in 6 per cent gum acacia and added to asparagine medium with final concentrations varying over a ten fold range of 0 1 to 1000 μ per milliliter of medium The tubes were inoculated with a spore suspension of *C immitis* and incubated at 35 C Observations made during a thirty day period showed complete inhibition of growth with concentrations of 100 μ per milliliter of medium and temporary suppression of growth with small quantities of drug

IN VIVO STUDIES

INFECTION

White male mice weighing approximately 25 gm were inoculated intraperitoneally with an 0 5 ml saline spore suspension of *C immitis* Determinations of the inocula were made in each experiment and found to vary from 100 to 700 spores

TREATMENT

All treatment was administered by subcutaneous injection

Mycostatin Two infected groups of 100 mice each were treated with Mycostatin beginning the day of infection One group received 0 5 mg and the other 1 0 mg daily for five consecutive days The animals were then allowed to rest for two days This schedule was followed for thirty days Fifty infected animals received physiologic saline

In Figure 1 are shown the results of this experiment In the control group animals began to die on the tenth day and by the eighteenth day 92 per cent were dead The mice which had received

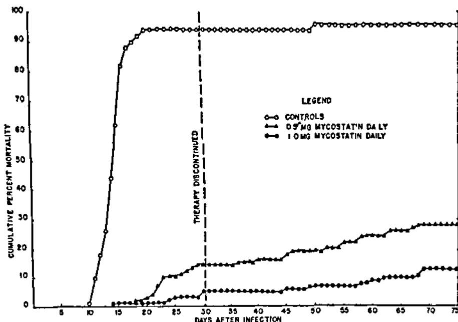


FIGURE 1

The treatment of coccidioidomycosis in mice with Mycostatin given immediately after infection

either 0 5 mg or 1 0 mg of Mycostatin showed a marked reduction in mortality (13 per cent and 28 per cent of deaths respectively) seventy five days after infection or forty five days after treatment was discontinued

In Figure 2 are the results of an experiment in which groups of 25 mice received 1 0 mg of Mycostatin for thirty days beginning at varying times after infection Over 90 per cent of the infected non treated animals were dead twenty nine days after infection Animals which had been started on treatment four six or eight days after infection showed a favorable response to the treatment When treatment was delayed beyond eight days the reduction in mortality was less striking

Aminostilbamidine The effect of aminostilbamidine treatment alone or in combination with Mycostatin was studied In this study treatment was started the day of infection These results are presented in Figure 3

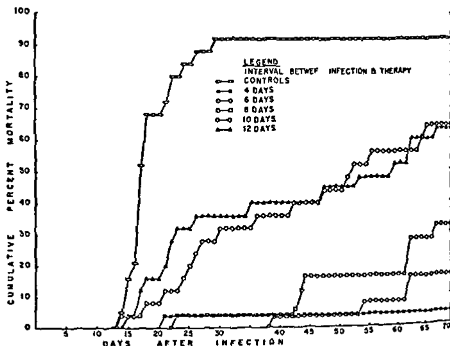


FIGURE 2

The effect of delayed Mycostatin therapy on coccidioidomycosis in mice

All 50 infected mice which had received injections of 6 per cent gum acacia were dead twenty days after infection Of 100 mice which had received 1 0 mg of aminostilbamidine for thirty days 62 per cent were dead forty five days after treatment was discontinued

Another group of 100 animals in this study was treated with 1 0 mg of aminostilbamidine and 1 mg of Mycostatin for thirty days This combined therapy was not more beneficial than the 1 mg of Mycostatin treatment presented in an earlier experiment

In the last group of experiments treatment of infected animals was delayed until the earliest sign of severe coccidioidomycosis (The results of these experiments are shown in Figure 4) The onset of deaths with extensive lesions of the lungs and viscera was the criterion used for beginning therapy On the fourteenth day after infection, 8 of 100 animals designated as controls 7 of 100 mice designated to receive 2 0 mg of Mycostatin and 2 of 100 mice

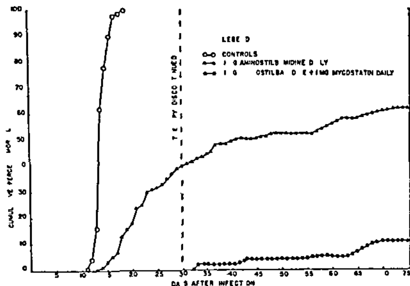


FIGURE 3

The effect of Mycostatin and aminostilbamidine therapy on coccidioidomycosis in mice

designated to receive 2 0 mg of aminostilbamidine died so treatment was begun On the twenty sixth day of treatment the daily dose of Mycostatin was reduced to 1 0 mg

In this very vigorous test of withholding the drugs until the mice began to die the treatment was soon associated with a reduction in the mortality rate However the mice continued to die

Although Mycostatin and aminostilbamidine delayed the course of infection as indicated by the mortality curves it was found that mice which had died during the course of treatment had extensive pulmonary and visceral lesions The animals sacrificed at the conclusion of the experiments had equally extensive lesions of the viscera but fewer pulmonary lesions These lesions contained many spherules

While there is no evidence that the infection has been eradicated these experimental results are encouraging in that these drugs delayed deaths and reduced the mortality during the period of observation However the search for additional antimicrobial drugs for coccidioidomycosis should continue

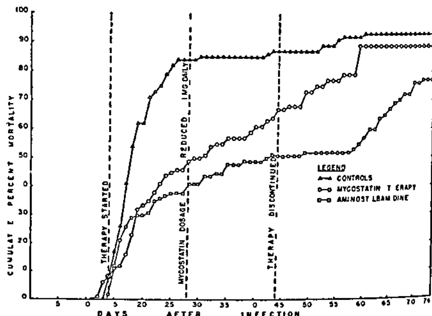


FIGURE 4

The effect of Mycostatin and aminostilbamidine therapy on severe coccidioidomycosis in mice

REFERENCE

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CHARLOTTE C CAMPBELL

Therapeutic Activity of Mycostatin in Mice Infected with *Histoplasma capsulatum, Coccidioides immitis, Cryptococcus neoformans, Candida albicans, or Sporotrichum schenckii*

Since the report of the discovery of Mycostatin in 1950 a number of investigators have shown that this antibiotic exerts a marked beneficial effect on the course of experimental systemic mycotic infections in animals The present report is a summary of the studies that have been carried out in this laboratory with Mycostatin * in mice infected with *Histoplasma capsulatum Coccidioides immitis Sporotrichum schenckii Cryptococcus neoformans*, or *Candida albicans*

Male Swiss albino mice (O Grady strain) weighing 18 to 20 gm were infected by the intravenous route with these agents suspended in cold physiologic saline solution Each of the strains employed was isolated from human sources The inoculating suspensions as described in earlier reports were standardized spectrophotometrically or as in the case of *C immitis* by dilution of a washed stock suspension of spores so that the 0 2 ml inoculum per mouse produced a mortality of at least 60 per cent by the fourteenth day in untreated animals Except for *C immitis* where the stock suspension was made from filamentous cultures grown on Sabouraud s agar for fourteen days at room temperature and maintained at 4 C for a series of experiments the inoculating suspensions were prepared fresh for each experiment from forty eight to seventy two hour yeast phase cultures grown on brain heart infusion agar at 37 C Only slightly soluble in water the drug was employed in

* The several lots of Mycostatin (HV-1 HA- 49-H HV-340-1F and HA-337-A) used in these studies were supplied by E R Squibb & Sons

aqueous suspensions prepared immediately before use and administered subcutaneously Single doses ranging from 0 5 to 3 0 mg were contained in 0 5 ml and were given in the various regimens described in Tables 1 through 6 Noninfected animals treated with each concentration of drug and infected but untreated mice were included in each experiment

Evaluation of therapeutic activity was based on the prolongation of survival time of treated as compared with untreated animals Except where otherwise noted experiments usually were terminated after twenty eight days

RESULTS

In animals infected with *H capsulatum*, a considerably higher percentage of those treated with Mycostatin (Lot HV-I) either at the time of infection or one week later survived than did the untreated controls This is illustrated in Table 1 which shows that

TABLE 1

THERAPEUTIC EFFECT OF MYCOSTATIN (LOT HV-1) IN EXPERIMENTAL HISTOPLASMOSIS (STRAIN G-8)

Group	No of Mice	Dosage (mg)	Drug Regimen*	Total Drug(mg)	Percent Survival on Day 14	28
1	20	-	-	-	30	0
2	20	1 0	A	7	100	95
3	20	0 5	A	3 5	85	55
4	20	1 0	B	6	75	40
5	20	0 5	B	3	70	35

*Regimen A At time of infection and twice daily for 3 days
" B Delayed Twice daily on 7th, 8th and 9th days.

35 to 95 per cent of the treated animals survived the twenty eight day observation period This is in contrast to a 100 per cent mortality by the twenty first day in untreated controls (group 1) As would be anticipated the survival percentage was highest in animals in which therapy in adequate dosage was started at the time of infection (group 2) However there was also some protection in

TABLE 2

THERAPEUTIC EFFECT OF MYCOSTATIN (HA 337-A) IN EXPERIMENTAL CRYPTOCOCCOSIS

Group	No of Mic	Dosage (mg)	Drug Regimen*	Total Drug(mg)	Per cent Survival on Day 14	28
1	40	-	-	-	27 5	0
2	40	0.25	A	1 75	96 0	20 0
3	40	0 5	A	3 5	100 0	72 5

* Regimen A At time of infection and at six approximately 12-hour intervals thereafter

the groups in which the dosage was inadequate (group 3) or therapy was delayed (groups 4 and 5)

Similar types of experiments carried out with Mycostatin (Lot HA-337-A) and mice infected with *C neoformans C immitis* or *C albicans* reveal that the drug also has a marked therapeutic effect in these experimentally induced diseases The action of the drug in sporotrichosis was less dramatic These studies as well as the dosages and regimens employed are illustrated in Tables 2 (cryptococcosis) 3 (coccidioidomycosis) 4 (candidiasis) and 5 (sporotrichosis) It will be noted that the maximum total dosages required for good protection in the different infections ranged from 3 5 to 10 mg This indicates that optimum regimen and

TABLE 3

THERAPEUTIC EFFECT OF MYCOSTATIN (HA-337-A) IN EXPERIMENTAL COCCIDIOIDOMYCOSIS

Group	No of Mice	Dosage (mg)	Drug Regimen*	Total Drug(mg)	Per cent Survival on Day 14	28	40
1	40	-	-	-	65	10	2 5
2	40	1 0	C	10	100	77 5	62 5
3	40	1 0	D	10	95	67 5	47 5

* Regimen C Daily for 10 days beginning with time of infection

D Daily for 10 days beginning 72 hours after infection

TABLE 4

THERAPEUTIC EFFECT OF MYCOSTATIN (HA-337-A) IN EXPERIMENTAL CANDIDIASIS

Group	No of Mice	Dosage (mg)	Drug Regimen*	Total Drug(mg)	Percent Survival on Day 14	28	40
1	20	-	-	-	20	0	0
2	20	0 5	C	5 0	80	70	60
3	20	1 0	C	10 0	95	80	70
4	20	1 0	D	10 0	77 5	60	65

* Regimen C Daily for 10 days, beginning with time of infection

" D Daily for 10 days, beginning 72 hours after infection

dosage in human cases of these diseases might vary also not only with the type and degree of infection but also with the causative agent

The maximum total dosage of 10 mg (500 mg /kg) given in 1 0 mg *daily doses of Lot HA-337-A used in most of the* experiments reported herein was well tolerated However there is evidence that the maximum tolerable level as well as therapeutic ac

TABLE 5

THERAPEUTIC EFFECT OF MYCOSTATIN (HA-337-A) IN EXPERIMENTAL SPOROTRICHOSIS

Group	No of Mice	Dosage (mg)	Drug Regimen*	Total Drug(mg)	Percent Survival on Day 14	21
1	40	-	-		12 5	0
2	40	0 5	A	3 5	55 0	2 5
3	40	1 0	A	7 0	67 5	5 0
4	40	1 0	C	10 0	92 5	27 5
5	40	1 0	D	10 0	95 0	20 0

* Regimen A At time of infection and twice daily for 3 days

" C Daily for 10 days, beginning with time of infection

" D Daily for 10 days, beginning 72 hours after infection

tivity varies appreciably from lot to lot As shown in Table 6 1 0 mg daily doses for ten days of Lot HV-340-1F protected 79 per cent of the animals infected with *H capsulatum*, whereas similar regimens with Lots HA-242-H and HA-337-A were considerably

TABLE 6

COMPARISON OF THERAPEUTIC ACTIVITY OF DIFFERENT LOTS OF MYCOSTATIN IN MICE INFECTED WITH *HISTOPLASMA CAPSULATUM*

Experiment	Mycostatin Lot No.	No in Group	P rc nt Survival on Day 14	28
1	HV-340-1F	40 - untreated	40 0	5 0
		40 - treated*	97 5	80 0
2	HA-242-H	30 - untreated	29 0	10 0
		30 - treated*	80 0	53 0
3	HA-337-A	20 - untreated	35 0	5 0
		20 - treated*	60 0	35 0

* 1 0 mg at time of infection and daily for 9 days

less effective The increased percentage of deaths in treated groups 4 and 6 by the fourteenth day also suggests that 10 mg total dosages of the latter two lots approached or exceeded the tolerable level for sick animals whereas the similar dosage of Lot HV-340-1F did not Such variation of course is a common experience in the development of any new drug Nevertheless it emphasizes the importance of employing only those lots with maximum activity for trial in human cases

VICTOR D NEWCOMER EDWIN T WRIGHT, THOMAS H STERNBERG, JAMES H GRAHAM, ROBERT H WIER and ROGER O EGEBERG

Evaluation of Nystatin in the Treatment of Coccidioidomycosis in Man

Nystatin an antifungal antibiotic has recently been isolated by Brown and Hazen[1] from cultures of *Streptomyces noursei* They have presented its chemical biologic and fungicidal properties in subsequent articles[2,3] In amounts of 1 56 to 6 25 μg /ml of broth it was found to inhibit effectively the growth of a large variety of nonpathogenic and pathogenic fungi including *Coccidioides immitis* In a more detailed study[4] we have evaluated the effect of nystatin both in vitro and in mice against *C immitis* Nystatin was found to have an inhibiting effect on the growth of *C immitis* in broth cultures in as low a concentration as 1 56 μg /ml and had a fungicidal effect in a concentration of 6 25 μg /ml The course of experimental coccidioidomycosis in mice was significantly altered when they were treated parenterally with nystatin This was manifested by longer periods of survivability and in many instances apparent biologic cures These observations were particularly striking in those infected animals in whom treatment was begun early Such favorable results associated with minimal evidence of toxicity led us to consider nystatin as a possible agent for the treatment of coccidioidomycosis in humans and this paper summarizes our experience to date

ORAL ROUTE

Nystatin was first given orally to a series of 10 patients with disseminated coccidioidomycosis It was administered in capsules or tablets in divided doses four times daily Treatment was continued

for periods as long as six months and in dosages as large as 12 gm daily No serious side reactions were observed necessitating discontinuance of the drug and no abnormal laboratory studies were encountered which could be attributed to the administration of nystatin Transitory nausea and diarrhea were occasionally encountered with the higher dosages These symptoms either ceased spontaneously or disappeared when the dosage was lowered Blood levels were ascertained periodically throughout the course of treatment and specimens were taken two to six hours after the administration of the drug These were determined by the microbiologic procedure described by Tarbet and Sternberg[8] In no instance were sustained or high therapeutic values obtained in the blood of any of the patients Clinically only one patient showed any improvement This patient is reported below in detail

Case 1 T P a 12 year old man entered the hospital on September 30 1951 with a history of the onset of swelling and tenderness in the right flank and right lower quadrant of the abdomen of nine months duration An extensive psoas abscess was discovered at surgery A biopsy of the abscess wall and cultures of the purulent material later revealed the presence of *C immitis* The coccidioidin skin test was 3 plus in a 1 1000 dilution and the complement fixation test was positive in a dilution of 1 4 Two fistulas in the right flank persisted following surgery and intermittently drained purulent material and at times undigested food particles A barium enema demonstrated a cecocutaneous fistula An ileotransverse colostomy was then done and later a right colectomy The right flank sinuses persisted and numerous minor surgical procedures were done over the following two years without improvement The patient then developed pain and tenderness in the right hip and lytic lesions in the right greater trochanter the head of the femur and the crest of the ileum were found on roentgenologic examination

At the time nystatin therapy was begun the patient s weight was 126 pounds and there was considerable limitation of motion in the right hip Nystatin was first given in a dosage of 0 5 gm daily and was gradually increased over a four month period to 12 gm daily This dosage was continued for two months During this time the sinus tracts healed the soft tissue swelling of the right hip subsided and considerable improvement in function of the right hip

was observed Roentgenologic examination of the right hip revealed a regression of the previous lytic lesions The patient also experienced a feeling of well being his appetite increased, and he gained 42 pounds

The patient was discharged from the hospital on August 20 1954 after receiving nystatin for approximately six months Recent follow up studies have shown no evidence of active disease

INTRAMUSCULAR ROUTE

Two patients with disseminated coccidioidomycosis who had previously received nystatin orally were given a preparation of nystatin intramuscularly Initially 25 000 units of nystatin were given four times a day and this dosage was gradually increased until both patients were receiving 200 000 units twice daily Blood levels were determined at periodic intervals and they varied from zero µg /cc of serum to 3 0 µg /cc of serum when the daily dosage was 400 000 units When 200 000 units of nystatin were given twice daily both patients developed chills fever, malaise and marked pain and tenderness at the injection sites Various amounts of hydrocortisone hyaluronidase and procaine hydrochloride were injected along with the nystatin in an effort to prevent the severe local reactions However this was unsuccessful The intramuscular route of administration of nystatin was discontinued because of the severe local reactions and the low blood levels obtained.

INTRAVENOUS ROUTE

Two intravenous preparations of nystatin have been made available to us for clinical use The first nystatin hydrochloride was given an extensive trial in one patient in whom very favorable results were obtained

Case 2 E S a 33 year-old Negro was admitted to the hospital on November 23 1953 One month prior to entry he had developed chills fever cough and chest pain Roentgenologic examination of the chest demonstrated a bilateral pneumonitis Penicillin was given without benefit and subsequently he developed hemoptysis subcutaneous abscesses and progressive weakness and lost approximately 50 pounds

The physical examination on admission revealed a chronically ill appearing thin poorly nourished Negro weighing 137 pounds Several large subcutaneous abscesses were present on the trunk and extremities A diagnosis of coccidioidomycosis was made and *C immitis* was subsequently recovered from the sputum and several of the subcutaneous abscesses On December 18 1953 the precipitation test for *C immitis* was 4 plus in undiluted serum but negative in dilutions of 1 10 and greater The complement fixation test for *C immitis* was positive in dilutions as high as 1 64 and on January 27 1954 it was 4 plus in a dilution of 1 128 The coccidioidin skin test was 2 plus in a dilution of 1 1000

The patient was given nystatin orally for approximately six weeks in a dosage as high as 16 gm daily During this time he continued to have a daily temperature of 102°F and additional subcutaneous abscesses developed which required repeated aspirations He subsequently developed lytic lesions in the left clavicle and left ileum

Nystatin was administered daily intravenously from March 12 to 28 the dosage being 200 000 units four times a day Blood levels varied from 10 to 18 μg /ml during the six hour period following the first 200 000 units injection Therapy was discontinued on March 28 because of the lack of additional drug The initial injections were accompanied by severe shaking chills and fever as high as 102 F These reactions subsided within one to three hours and did not necessitate discontinuance of therapy

Intravenous treatment with nystatin was reinstituted on April 12 Because of the sclerosing effect which it had on the superficial veins it was necessary to insert a polyvinyl tube into a deep vein and administer nystatin through it It was necessary to insert the polyvinyl tube in several additional veins because of the development of thrombophlebitis A daily dosage of 200 000 units four times a day was given until August 8 at which time it was discontinued due to the technical difficulties encountered in administering the drug

During the early weeks of treatment the patient developed a roentgenologic picture of miliary pulmonary coccidioidomycosis which completely cleared in one month At the completion of treatment he had gained 38 pounds most of the subcutaneous abscesses had disappeared and the rest had decreased in size and repeat

roentgenologic examination of the previously affected bones showed no increase in the lytic areas He had been afebrile for sev eral weeks The patient left the hospital against medical advice and a follow up has not been obtained

During the course of treatment numerous blood levels for ny statin were determined These were in the range of 40 to 45 μg /ml

In an effort to detect any toxic effect of the drug a large number of laboratory procedures were performed throughout the period of treatment No abnormalities were encountered which could be attributed to the drug

Recently we have obtained another preparation of nystatin for intravenous use This preparation is said to be more stable and of greater purity [6] and our clinical experience to date indicates that its sclerosing effect on the veins is much less

We are now treating three patients with disseminated coccid ioidomycosis with this latter preparation One patient has been treated for a period of two months and is reported in detail

Case 3 T K a 31 year old Negro first developed sudden onset of chills fever and general malaise on January 1 1954 This was accompanied by pleuritic pain in the left chest and a mild cough with hemoptysis Two weeks later x ray examination of the chest revealed a right lower lobe bronchial pneumonia One month fol lowing the onset of illness the patient entered the hospital with an exacerbation of his previous symptoms and in addition a history of a 10 pound weight loss

Physical findings revealed a chronically ill young man No other significant findings were present No disease was found on roent genologic examination of the chest Acid fast bacilli were not found on examination of the sputum The sedimentation rate was moder ately elevated The coccidioidin skin test was 2 plus with the use of a 1 1000 dilution and the complement fixation test for *C immitis* was 1 plus in a 1 4 dilution

While the patient was in the hospital an abscess developed in the right deltoid area This was aspirated and microscopic examination of the purulent material revealed the presence of spherules of *C immitis* A pure culture of *C immitis* was obtained from this material Later an additional abscess developed in the left anterior axillary fold which opened and drained purulent material contain

ing the spherules of *C immitis* About this same time a lytic area was demonstrated by roentgenologic examination to be present in the anterior portion of the right eighth rib

Nystatin was initially given on October 10 and at this time the complement fixation had risen to 4 plus in a 1 64 dilution The drug was given via the intramuscular route in a dosage of 100 000 units four times a day for approximately three weeks Severe pain and tenderness developed at the sites of the injections and it was necessary to discontinue the drug

The titer of the complement fixation test gradually dropped during the following five months so that by March 22 1955 it was 4 plus in a 1 8 dilution However the cutaneous abscesses continued to drain intermittently

Treatment with the new intravenous preparation of nystatin was begun on April 14 This was administered in a dosage of 100 000 units in 1000 cc of 5 per cent glucose in water over a four hour period This has been gradually increased until at the present time the patient is receiving 400 000 units daily without any evidence of toxicity The two abscesses have healed The lytic area in the previously affected eighth rib has remained unchanged

Several blood levels for nystatin were obtained and these have been as high as 15 µg /ml three hours after the administration of 200 000 units The only side reaction observed to date was the development of chills and fever one hour after the first administration of the drug

The most recent complement fixation was 4 plus in a 1 4 dilution and the patient shows no evidence of active disease

DISCUSSION

This is a preliminary report regarding the use of nystatin in the treatment of coccidioidomycosis in man and only general observations can be made The drug is poorly absorbed when administered via the oral route With the use of relatively large doses over a long period of time apparently enough was absorbed to influence favorably the course of coccidioidomycosis in the one patient who was reported in detail No toxicity other than transient nausea and diarrhea was noted

The intramuscular preparations which we used were unsatis

factory because of the development of severe pain and tenderness at the site of injection

The intravenous route of administration appeared to be the most satisfactory in that the highest blood levels of nystatin have been obtained The first intravenous preparation was unsatisfactory in that it produced thrombophlebitis and ultimate sclerosis of the veins and this occurred even when the drug was administered through polyvinyl catheters placed in large veins The one patient who was treated responded dramatically and is in a period of remission The most recent intravenous preparation appears to have a much less irritating effect on the veins and indwelling catheters have not been required The patient who is presently receiving the latest intravenous preparation has shown a similar response

No serious side reactions necessitating the discontinuance of the drug have been noted with the use of the most recent intravenous preparation in the dosages employed to date Laboratory studies have failed to demonstrate any evidence of toxicity

With the initial administration of intravenous nystatin Herxheimer like reactions have been observed These consisted of chills fever up to 102°F and general malaise beginning approximately one half hour to one hour after starting treatment The reactions usually subside within four to six hours Similar reactions were observed with the second and third injections but were milder in character

No definite conclusions can be drawn with regard to the ultimate therapeutic value of nystatin in the treatment of coccidioidomycosis in man However we do feel that nystatin merits further clinical evaluation in the treatment of this disease

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I B CHRISTISON, N F CONANT AND
C K BRADSHER

The Sensitivity of *Histoplasma capsulatum* to Rhodanine and Related Compounds*

Organic sulfur compounds, especially dithiocarbamic acid derivatives, have been used profitably in agriculture as fungicides and insecticides for a number of years [1] Infrequent reports of their possible value as chemotherapeutic agents in human fungus infections could be found in the literature [2, 3] The activity of these compounds has been ascribed to the presence of the dithiocarbamate linkage, —N—C(=S)—S— In other known antifungal compounds such as the chalcones, acrylophenones, clavacin, and penicillic acid, the activity has been ascribed to an α, β unsaturated carbonyl linkage, —C=C—C=O [4]

Compounds containing the dithiocarbamate as well as the α, β unsaturated carbonyl linkage might be expected to exhibit antifungal activity Rhodanine contains —N—C(=S)—S— as part of its ring structure and undergoes condensation with aldehydes to yield compounds which also contain the —C=C—C=O linkage Several such rhodanine derivatives have been synthesized [5, 6] and tested for their mildew preventing activity [7-9] Some of these compounds were tested for antifungal activity against *Histoplasma capsulatum*

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MATERIALS AND METHODS

A yeast phase stock culture of *H capsulatum*, #1071, was maintained on paraffin sealed, brain heart infusion blood agar slants at 37°C The inhibitory effect of the compounds on yeast phase cultures of *H capsulatum* was assayed in Salvin s liquid medium at 37°C (Salvin [10]) Duplicate samples of each compound were added to 75 ml of the above medium in each of two 250 ml Erlenmeyer flasks In the lower dilutions the compounds were weighed and added directly to the medium (for example 15 mg /75/ml = 200 ppm) For the higher dilutions it was necessary to dissolve the compounds in propylene glycol from which the proper dilutions could be made Also, one of the duplicate flasks containing a compound previously dissolved in propylene glycol was heated for five minutes in the autoclave to increase solubility Control flasks contained medium only and medium with propylene glycol in the amount used for dissolving the compound

All cultures were examined microscopically on the fourth day and growth was recorded as present (+) or absent (−)

The following compounds were tested for antifungal activity rhodanine, 3 substituted 5 substituted 3 5 substituted rhodanine derivatives as well as 2 thiazolidinethione, 2,4-dioxothiazolidine and tetraethylthiuram disulfide

RESULTS

A preliminary assay of antifungal activity of rhodanine against five fungi was made (Table 1)

Table 1 shows that rhodanine was more active against *H capsulatum* than against any of the other fungi *H capsulatum* was selected, therefore to measure changes in antifungal activity caused by substitutions on the rhodanine nucleus

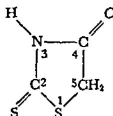
The effect of substituting $>CH_2$ for the $>C{=}O$ in the 4-position and $>C{=}O$ for the $>C{=}S$ in the 2 position on the rhodanine nucleus is presented in Table 2

Table 2 shows that these substitutions greatly reduced the antifungal activity of the rhodanine moiety Such results indicate that

TABLE 1

ANTIFUNGAL ACTIVITY OF RHODANINE

1	Histoplasma capsulatum	10 ppm*
2	Blastomyces dermatitidis	200 ppm
3	Candida albicans	> 200 ppm
4	Cryptococcus neoformans	> 100 ppm
5	Trichophyton mentagrophytes	200 ppm



* Parts per million giving complete inhibition
1 Salvin's medium 37 C yeast phase
2 Sabouraud's agar 37 C yeast phase
3 Sabouraud's agar 37 C.
4 Sabouraud's agar 37 C
5 Sabouraud's agar room temperature

the activity of rhodanine might depend equally on >C=S and =C–C=O

Rhodanine undergoes condensation with aldehydes to yield compounds with substitutions at the 5 position The activity of eight such rhodanine derivatives is presented in Table 3

TABLE 2

ACTIVITY OF RHODANINE AND RELATED COMPOUNDS AGAINST *HISTOPLASMA CAPSULATUM*

Rhodanine	2 thiazolidinethione	2,4-dioxothiazolidine
10 ppm	200 ppm	> 200 ppm

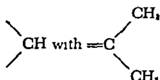
TABLE 3

ANTIFUNGAL ACTIVITY OF SOME 5-ALKYLIDENE DERIVATIVES OF RHODANINE AGAINST *HISTOPLASMA CAPSULATUM*

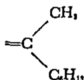
HN—C(=O)—C(=R)—S—C(=S)—N

Compound No	R	
223	$=C(CH_3)(CH_3)$	200 ppm
233	$=C(CH_3)(C_2H_5)$	200 ppm
247	$=C(CH_3)(C_3H_7)$	50 ppm
261	$=C(CH_3)(C_5H_{11})$	200 ppm
239	$=C(CH_3)(C_6H_{13})$	10 ppm
255	$=C(C_2H_5)(C_2H_5)$	50 ppm
260	$=CH—CH(C_2H_5)(C_4H_9)$	5 ppm
244	$=C<(CH_2—CH_2)(CH_2—CH_2)>CHCH_3$	50 ppm

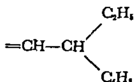
The compounds in Table 3 contain $-\overset{|}{N}-\overset{S}{\overset{\|}{C}}-S-$, the dithiocarbamate system, and also have an α, β unsaturated carbonyl system, $-\overset{|}{C}=\overset{|}{C}-\overset{|}{C}=O$, as in clavacin, penicillic acid, etc In mildew prevention tests with this group of compounds, Brown et al [8] reported an increase in antifungal activity with an increase in the length of the carbon chain up to six carbons Such a generalization could not be made from the results of tests with *H capsulatum* However, the activity lost by replacing



at the 5 position was regained when the substitution was



and, also, activity was greater than that of rhodanine when



was the substituent

Condensation of rhodanine with various aromatic aldehydes gave a series of benzylidene rhodanine derivatives The activity of seven such compounds is presented in Table 4

Table 4 shows that benzylidene rhodanine as well as the phenolic compounds and an alkyl derivative exhibited the same or slightly better activity than unsubstituted rhodanine It is interesting to note that inhibition by the halogen derivatives was related to the position of Cl on the benzine ring When chlorine was substituted at the ortho and meta positions, the compounds showed excellent inhibitory activity Substitution at the para position however, greatly reduced the activity of the compound

TABLE 4

ANTIFUNGAL ACTIVITY OF SOME 5-ARYLIDENE DERIVATIVES OF RHODANINE AGAINST *HISTOPLASMA CAPSULATUM*

Compound No	R	
159	=CH–C_6H_5	10 ppm
209	=CH–C_6H_4–CH_3	5 ppm
200	=CH–C_6H_4–Cl	1 ppm
366	=CH–C_6H_4–Cl	5 ppm
290	=CH–C_6H_3(R)–Cl	200 ppm
193	=CH–C_6H_4–OH	10 ppm
292	=CH–C_6H_3(OH)–Cl	5 ppm

The activity of compounds with substitutions on the nitrogen at the 3 position is presented in Table 5

In Table 5 all of the compounds tested with the exception of 3(p carboxyphenyl) rhodanine demonstrate excellent inhibitory activity Substitutions of an alkyl radical and the halogens Cl and Br at the para position of the phenyl ring increased the activity over that shown by 3 phenylrhodanine 50 ppm The substitution

TABLE 5

ANTIFUNGAL ACTIVITY OF 3-SUBSTITUTED RHODANINES AGAINST *H CAPSULATUM*

R—N—C(=O)—CH_2—S—C(=S)—N (rhodanine ring)

Compound No	R	
370	$-C_6H_5$	50 ppm
466	$-C_6H_4-CH_3$	10 ppm
396	$-C_6H_4-Cl$	10 ppm
454	$-C_6H_4-Br$	10 ppm
393	$-C_6H_4-C(=O)-OH$	200 ppm
391	$-CH(CH_2-CH_2)_2CH_2$	5 ppm

of a carboxyl group in the para position resulted in a greatly decreased inhibitory effect 200 ppm Greatest activity occurred in tests with 3 cyclohexylrhodanine, 5 ppm

Rhodanine with substitutions at both the 3 and 5 positions was tested for activity against *H capsulatum* The results of these tests are presented in Table 6

Table 6 shows that the substitution of $-CH_3$ for —H in the 3 position reduced the activity of 5 benzylidene rhodanine (159, Table 4) from 10 ppm to > 200 ppm A similar substitution on p methyl 5 cyclohexylidene rhodanine (244, Table 3) reduced its activity from 50 ppm to > 200 ppm In a similar manner, the activity of 5(methylethylidene) rhodanine (223, Table 3) was changed from 200 ppm to > 200 ppm It would seem that the size of the molecule and/or its inability to be tautomerized might make the 3,5-disubstituted rhodanines less active

Many of the more active compounds discussed above were tested

TABLE 6

ANTIFUNGAL ACTIVITY OF SOME 3,5-DISUBSTITUTED RHODANINE DERIVATIVES AGAINST *HISTOPLASMA CAPSULATUM*

Compound No	*R*	*R'*	
354	$—CH_3$	$=C(H)—C_6H_5$ (=CH—phenyl)	> 200 ppm
355	$—CH_3$	$=C<(CH_2—CH_2)_2>CHCH_3$	> 200 ppm
420	$—CH_2—C_6H_5$ (—CH₂—phenyl)	$=C(CH_3)_2$	> 200 ppm

General structure: R—N, ring N—C(=O)—C(=R')—S—C(=S)—N (3-R-5-R'-rhodanine)

for acute and chronic toxicity in mice in preparation for in vivo protection tests against experimental histoplasmosis in these animals Unfortunately the compounds tested proved to be toxic. Furthermore, when some of the more active compounds were tested in the presence of 20 per cent human serum their activity was reduced greatly

Due to the remarkable inhibitory activity of rhodanine and its various derivatives against *H capsulatum* a search was made for other compounds of known pharmacologic activity which contained the $—N—C(=S)—S$ structure Because the pharmacologic behavior of tetraethylthiuram disulfide (antabus) is known [11] and

its antifungal activity is not greatly reduced in the presence of whole blood,[2] this compound was tested for its antifungal activity The results of these tests are presented in Table 7

TABLE 7

ANTIFUNGAL ACTIVITY OF TETRAETHYLTHIURAM DISULFIDE AGAINST FIVE PATHOGENIC FUNGI

Histoplasma capsulatum	5 ppm
Blastomyces dermatitidis	200 ppm
Candida albicans	100 ppm
Trichophyton mentagrophytes	100 ppm

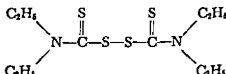


Table 7 shows that tetraethylthiuram disulfide inhibited the growth of *H capsulatum* at 5 ppm The other three fungi tested were inhibited only at much higher concentrations of the compound

The effect of tetraethylthiuram disulfide on experimental histoplasmosis in mice was investigated White Swiss mice weighing 20 gm were inoculated intraperitoneally with 0 5 ml of a 1 100 suspension of a three day old yeast phase culture in 5 per cent hog gastric mucin

Dosage was determined on the basis of the minimal effective in vitro concentration of the compound Two three fold dilutions above and one three fold dilution below this level determined doses of 45 15, 5 and 1 7 mg /mouse/day The individual doses were divided, half being given in the morning and half in the afternoon Treatment by intraperitoneal injection was begun on the afternoon of the first day and continued for the next four days Drug controls were included in the experiment (Table 8)

Table 8 shows that intraperitoneal inoculation of tetraethyl thiuram disulfide, at the levels used, did not protect mice against experimental histoplasmosis

TABLE 8

PROTECTION OF MICE INFECTED WITH *HISTOPLASMA CAPSULATUM*

DRUG TETRAETHYLTHIURAM DISULFIDE (ANTABUS) *

Day of Experiment		*Number of Mice Surviving*				
		Amount of Drug in mg/dose/day †				
		45	*15*	*5*	*17*	*0*
Infected mice	1	5	5	5	5	5
	2	5	4	4	4	5
	7	4	4	4	4	5
	8	4	4	3	4	4
	9	4	3	3	2	4
	11	1	0	1	1	4
	14	0	0	1	1	3
	21	0	0	1	1	2
Drug control	1	5	5	5	5	
	14	5	5	5	5	
	21	5	5	5	5	

* Complete in vitro inhibition at 5 ppm
† Drug given morning and afternoon for 5 days

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S B SALVIN and EDGAR RIBI

Structural Elements of *Histoplasma capsulatum* and Their Role in Immunization against Experimental Disease

Immature male white Swiss mice were protected against a lethal intracerebral challenge with *Histoplasma capsulatum* by prior intraperitoneal inoculation with killed whole cells of the yeast phase The immunization effected a reduction in the numbers of viable fungus cells in the liver and spleen and in other tissues associated with the site of injection

This immunizing activity was examined to determine whether it was located in the capsule the cell wall or the internal protoplasm The capsule was eliminated as a possible source of protective antigen since neither a true capsule nor a fine slime layer could be detected with the aid of the electron microscope This fact became evident after comparative studies of capsulated and noncapsulated fungi wherein a technique of specimen preparation was used that did not alter the original morphology of the organisms

A technique was devised for separating cell walls from the internal protoplasm This technique involved killing the cells exposing them to mechanical vibration and then by centrifugation washing out the protoplasm from the broken cells When these two cell fractions were tested in mice the immunizing antigen was detected only in the cell wall

PARKER R BEAMER, DALE M SCHULZ, DWAIN N WALCHER, and FRANK VELLIOS

Histoplasmosis Pathogenesis and Immunology in Relation to Therapy*

The specific effectiveness of antibiotics and chemical agents in the therapy of systemic histoplasmosis in man is difficult to appraise Infection may exist without apparent disease and clinical manifestations of the disease are protean probably owing to a complex interplay of several factors such as the biologic properties of the fungus the type of infection the distribution and size of lesions the immune status of the patient and the histologic features of the lesions Ordinarily histoplasmosis seems to be a benign infection with or without symptoms and signs of illness but a massive inoculum or poor resistance of the host or both may lead to slowly or rapidly progressive fatal disease[1]

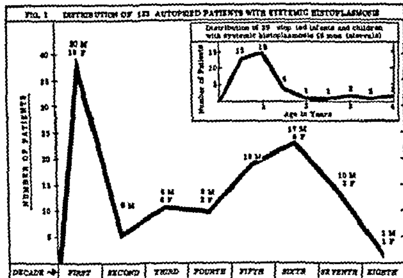
PATHOGENESIS

One approach to an understanding of the pathogenesis of histoplasmosis is to analyze the mortality curve and pathologic findings determined by postmortem examinations Much of the material for the present study consisted of 112 fatal cases selected from the literature[2] and 11 additional cases examined by us the choice of cases being based on availability of adequate data

Two peaks of mortality were noted one in the first decade of life and another during the fifth and sixth decades (Fig 1) The inset graph indicates that 72 per cent of deaths in the first decade occurred in infants twelve months old or less and both sexes were affected in essentially the same frequency During the fourth to

* We are indebted to Messrs James F Glore and Paris Johnson of the Department of Illustration Indiana University School of Medicine for preparation of the line drawings and graphs

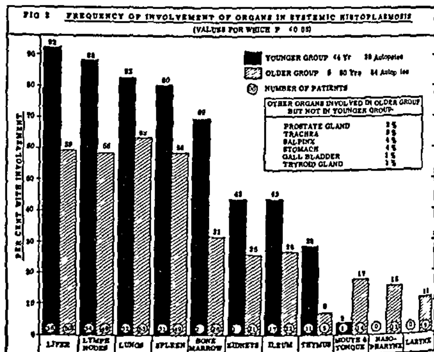
FIG. 1 DISTRIBUTION OF 123 AUTOPSIED PATIENTS WITH SYSTEMIC HISTOPLASMOSIS



seventh decades there was a striking predominance of the disease in males particularly in the fifth and sixth decades (36 men 6 women) Application of the chi square test indicates that this difference is statistically significant

Inasmuch as a break in the incidence of fatal infections occurred after four years of age the authors arbitrarily classified 39 patients as a younger group and 84 patients as an older group The liver lymph nodes lung spleen bone marrow kidney and ileum in decreasing order were involved more frequently in the younger patients than in the older (Fig 2) The chi square test was applied to these differences and the figures illustrated in the bar graph had a P value of 0 05 or less Thus the different frequencies of involvement of these organs seem to be statistically significant In addition certain other organs or tissues were affected more frequently in the younger group but these values are of doubtful significance the P value being more than 0 05 (Table I) The greater frequency of involvement of more organs or tissues in infants and young children indicates more widely disseminated disease in that group

In contrast to the above findings the buccal cavity (and tongue) nasopharynx and larynx were involved more frequently in the older group particularly men and the differences shown in the bar



graph (Fig 2) are of statistical significance according to the chi square test In addition certain other organs were involved exclusively in the older group (Fig 2 inset) but the number of patients was small and the differences are not statistically significant Although adrenal glands were affected with almost the same frequency in both groups there seemed to be a relatively greater tendency for this organ to be diseased in the older patients

Systemic histoplasmosis is a disease involving principally organs that *are richly* endowed with reticuloendothelial and lymphoid tissue, such as the liver lungs spleen lymph nodes and bone marrow The skin or gastrointestinal tract may have been the portal of entry in some instances but the skin was involved in a relatively small number of patients in this series and the frequency of lesions in the intestinal tract was only about half that observed in the lungs On the other hand the lung was the most frequently involved organ in the older group (63 per cent) and this frequency was exceeded only by lesions in the liver and lymph nodes in the younger group It seems pertinent that histoplasmosis of the buccal cavity (and tongue) nasopharynx and larynx was present al

TABLE 1 FREQUENCY OF INVOLVEMENT OF ORGANS IN HISTOPLASMOSIS (VALUES FOR WHICH P >0 05)

ORGAN	YOUNGER GROUP UP TO 4 YRS OLD 39 AUTOPSIES		OLDER GROUP 5 60 YRS OLD 84 AUTOPSIES	
	NO WITH LESIONS	PCT WITH LESIONS	NO WITH LESIONS	PCT WITH LESIONS
ADRENAL GLAND	18	46	43	31
COLON	13	33	21	25
SKIN & SUBCUT TISSUE	8	21	11	13
HEART	6	15	15	18
PANCREAS	5	13	7	8
BRAIN & MENINGES	2	5	9	11
APPENDIX	3	8	5	6
BLADDER	2	5	3	4
TESTIS	2	5	3	4
JEJUNUM	2	5	2	2
DUODENUM	2	5	1	1
ESOPHAGUS	1	3	1	1
PARATHYROID GLAND	1	3	1	1
PITUITARY CAPSULE	1	3	1	1
MIDDLE EAR	2	5	0	0
SKELETAL MUSCLE	1	3	0	0

most exclusively in the older group and such lesions were associated with one another to a significant degree

If these three sites were considered as a group and compared with the liver lung spleen lymph nodes and bone marrow as a group there was a significant degree of nonassociation Thus the data support the concept that the respiratory tract is the usual portal of entry infection of the upper respiratory tract may exist as a localized process in the older group becoming part of disseminated histoplasmosis later in the illness Furthermore the mode of inception and development of systemic histoplasmosis in the younger patients is probably different from that usually observed in older persons

REACTION TO INFECTION

Epidemiologic and clinical studies indicate that 50 to 80 per cent of persons in certain regions of the United States may have been or are infected with *Histoplasma capsulatum* and occasionally sporadic eruptive outbreaks are observed[8] Nevertheless deaths from histoplasmosis are relatively rare It seems likely that man has little ability to resist initial infection and that the organisms are not highly virulent for most persons Ordinarily the host

seems to acquire resistance during infection [4] and the lesion tends to heal, resulting in a calcified nodule

Studies on organs from fatal cases of histoplasmosis provide evidence of different types of tissue reactions related to the host's immune status and similar to those observed in first infection and reinfection tuberculosis Particularly in infants but sometimes in older persons the organisms multiply and disseminate widely parasitizing reticuloendothelial tissues in many parts of the body (Fig 3A) There is little if any evidence of reaction in the tissues In certain other instances in this group there is evidence of wide dis

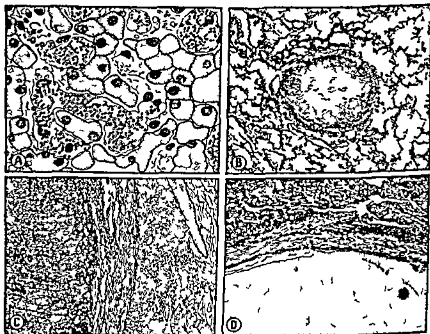


FIGURE 3

A. Sketch of liver with numerous small yeastlike organisms engorging large reticuloendothelial cells in the sinusoids between hepatic cells no inflammatory reaction noted

B Sketch of tubercle like granuloma in the lung with fibrosis beginning at the edge of the lesion

C. Sketch of adrenal gland with massive coagulative necrosis on the left and inflammatory reaction on the right

D Sketch of solitary nodule in the lung calcific flecks and numerous organisms are present in the caseous necrotic region in the lower half of the field a dense collagenous wall encloses the caseous focus

semination to many organs but the reticuloendothelial cells prolif erate and tubercle like granulomas are formed Some patients show even more evidence of resistance and fibroblasts proliferate about the periphery of the lesion (Fig 3B) The lesion sketched in Fig ure 3A is usually associated with a negative skin test and negative tests for circulating antibody whereas such tests are negative or equivocally positive in many patients with the tubercle like reac tion diagrammed in Figure 3B The tissue reactions and clinical courses observed in such patients are similar to those experienced in fatal first infection tuberculosis

Localized or less widely disseminated lesions usually observed in older persons provide evidence that tissues may acquire resistance similarly to the phenomenon in reinfection type tuberculosis On the other hand if infection is established in an organ the tissue reaction is usually violent resulting in greater degrees of coagula tive or caseous necrosis with associated inflammatory reaction at the periphery (Fig 3C) This sort of response is usually associ ated with hypersensitivity the histoplasmin skin test is positive in the majority of these patients but it may be negative during late stages of the disease

THERAPY

Partially healed relatively large solitary nodular pulmonary le sions have been noted in several patients Those found in fatal histoplasmosis of infants and young children probably represent the primary sites from which dissemination occurred during the process of healing Similar solitary nodules in the lung are ob- served in radiologic studies of older patients most of whom have little or no symptoms related to the lesion We studied solitary nodules removed surgically from 4 young adults who had positive reactions to histoplasmin and negative to tuberculin Histologic examinations revealed nodular foci of caseous necrosis with nu merous Histoplasma organisms enclosed within a wall of highly cellular or densely collagenous connective tissue (Fig 3D) Surgi cal removal of these lesions would seem effective in preventing pos- sible future dissemination of the disease in such patients

In our experience treatment of disseminated histoplasmosis in infants and children is discouraging Numerous chemical agents

and antibiotics have been unsuccessful In the past four years ethyl vanillate[6] was used for treatment of 7 infants and children with proved disseminated histoplasmosis and only 1 survived Apparently unavoidable severe toxic symptoms seemed to be correlated directly with the administration of ethyl vanillate in amounts sufficient to attain a blood level of 20 mg per 100 ml and in one instance serious imbalance of electrolytes occurred in a patient with a blood level of only 9 mg per 100 ml On the other hand we have observed apparent recovery from proved disseminated histoplasmosis in 8 infants and children who were treated only by nonspecific supportive measures In considering the use of certain chemotherapeutic agents in disseminated histoplasmosis one must consider judiciously the possibility that the drug may antagonize or counteract one or more of the several mechanisms that are significant in a patient s natural resistance

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CHARLES A BRASHER
and MICHAEL L FURCOLOW

Problems in Treatment of Chronic Histoplasmosis As Experienced in Over Twenty Cases

The problems presented by the treatment of the type of histoplasmosis most commonly seen in our group and requiring treatment — that is the chronic cavitary type — are largely the same as those encountered in the experimental treatment of chronic pulmonary tuberculosis It is evident therefore that a drug with marked therapeutic effect must be employed in order for the effect to be visualized with these chronic cases in whom the progress is ordinarily slow and fatal termination occurs only after years of observation

It is obvious that large numbers of cases must be observed and adequate controls set up in order to determine therapeutic effect Due to the chronic nature of the disease slight effects would obviously be missed Accordingly the Veterans Army Navy Therapeutic Conferences have been followed carefully and some preliminary thought has been given to setting up control series However due to the infrequency of these cases nothing beyond preliminary experiments involving attempts at finding a good therapeutic tool has been run The present series of cases does demonstrate the lack of either marked therapeutic effect or marked toxicity in any of the various therapeutic agents tried

The finding of chronic cavitary histoplasmosis in three different tuberculosis sanatoria in the area of high histoplasmin sensitivity indicates that this is a problem of sizable importance Between 7 and 8 per cent of the patients in these sanatoria demonstrated either proved histoplasmosis or positive serology suggesting active

disease Many of these cases are of the chronic cavitary type which in our experience is chronic progressive that is leads usually to dissemination and death The time required for dissemination may be some years

It has been estimated that there are probably as many as 1300 cases of histoplasmosis masquerading as or combined with tuberculosis in the various tuberculosis sanatoria in the high area Attempts have been made to encourage the finding of these cases and it is expected that a large pool of potential therapeutic cases will be available within the next year or so The importance of these trials therefore cannot be overstimated in setting up procedures for the treatment of these cases and outlining difficulties

MICHAEL L FURCOLOW
and CHARLES A BRASHER

Trials with 2-Hydroxystilbamidine, Aminostilbamidine, MRD–112, and Other Agents in Pulmonary Histoplasmosis, Experiments with More Than Twenty Cases

A total of 21 cases of pulmonary disease have been treated with various drugs during the past two years Most of the cases are of the advanced cavitary type of histoplasmosis The results are difficult to evaluate Due to difficulties of evaluation it was decided to set up protocols in an attempt to screen the drugs looking for marked improvement with any given drug Any favorable drugs uncovered would then be more carefully evaluated in a control method

It should be noted that a few of the patients were treated with more than one drug No one was treated with more than one drug at a time When no response was observed with an earlier drug other drugs were subsequently tried The total number of individuals involved in this report is 21

The first drug available to us was a compound 8 diethylamino ethyl fencholate (MRD–112) from the William S Merrell Company A total of 14 cases were treated with this drug — 12 of them had histoplasmosis and 2 had other diseases One of the latter cases had cutaneous and cerebral cryptococcus the other had tuberculous meningitis undiagnosed until post mortem Five of these 14 patients have expired 1 with tuberculous meningitis 1 with disseminated histoplasmosis and tuberculosis 1 with cavitary histoplasmosis who died following surgery and a six year old boy with disseminated histoplasmosis who died within two days after institution of treatment Another case had cryptococcus of the skin

which later became cerebral and the patient expired It must be stated that no marked effects of MRD-12 in suppressing fungus growth were noted However sufficient experience was acquired with this drug to know that it was also relatively nontoxic at least in the dosages we used The dosage employed on these patients was 150 mg daily Several of the patients also received combined oral therapy either before or after intravenous therapy Some difficulty was experienced in one or two patients with sclerosing of the veins

Four cases were treated with 2 hydroxystilbamidine (also supplied by William S Merrell Company) Three were patients with histoplasmosis and one had blastomycosis of the larynx and lungs The latter patient appeared to have a satisfactory recovery but died some time later apparently of cardiac disease although no autopsy was obtained The second patient with disseminated histoplasmosis died without any evidence of response to the drug He also suffered from arteriosclerotic heart disease and liver damage This was not connected with the use of 2 hydroxystilbamidine A third patient died following treatment but he had both tuberculosis and histoplasmosis The fourth patient was treated without marked effect Our experience with 2 hydroxystilbamidine also did not indicate any marked fungicidal effect

A total of 3 cases have received aminostilbamidine — all of these being cases of disseminated histoplasmosis of the cavitary type No toxic responses were observed and no definite therapeutic effect was observed All of the patients completed their treatment of sixty days without adverse effects

Two patients have been treated with nystatin supplied by the Squibb Institute One of these had histoplasmosis and the other an undiagnosed fungus disease of the lungs — probably blastomycosis No toxic effects have been observed nor has there been any marked evidence of therapeutic effect although it is probably too early to decide

One case has been treated with testosterone This patient had disseminated histoplasmosis and lesions on the tongue and has been treated with several other drugs He seems to show early response to the drugs and later relapses He has shown slow progression of the disease and lately a positive blood culture Testosterone does not seem to have had any marked effect This man also has been treated with aminostilbamidine and MRD-112

A patient has been treated with antabuse (tetraethyl X) made by Ayerst Laboratories This patient has been treated for thirty days and no toxic effects have been observed nor have any marked effects on the histoplasmosis been found The use of antabuse was suggested by Smith and Conant

SUMMARY

Twenty-one patients with histoplasmosis or other proved or suspect fungus diseases of the lungs have been treated with various chemicals or antibiotics in an attempt to set up technical procedures and to demonstrate if possible some marked therapeutic effect

The value of the present series lies largely in the absence of demonstrated toxicity with these various chemicals No significant therapeutic effect has been observed although this type of case — that is the bilateral cavitary case of chronic nature — is a difficult one in which to determine any except marked therapeutic effect

It is quite probable that therapy with these agents should be continued longer in these cases Our present plans are to attempt to continue treatment for ninety or even 180 days in these cases in the future as therapeutic effects are sometimes slow in appearing

R L MAYER, E KONOPKA, S GEFTIC, and J TANZOLA

Sulfonamides and Experimental Histoplasmosis

In view of the contradictory results of the influence of sulfonamides in histoplasmosis we have investigated the action of a number of sulfonamides by both the in vitro and in vivo techniques

IN VITRO EXPERIMENTS

The anti sulfonamide action of twenty sulfonamides was tested in fluid Sabouraud s medium and synthetic medium free of PABA The organism grew more slowly in the synthetic medium than in Sabouraud s medium but the amount of growth was ultimately identical in both media A number of experiments were made with the synthetic medium to which 2 ml per cent of beef serum was added In this medium the organism grew at 37°C in the yeast phase throughout the experiment

RESULTS

The results obtained with these two media were virtually identical and we shall therefore report only the results obtained with the synthetic medium (Table 1)

The lowest concentration in which complete fungistatic activity was obtained with any of the sulfonamides tested was 1 1000 However a few compounds for example sulfapyridine sulfapyrazine and sulfadiazine strongly retarded growth even at a concentration of 1 10 000 Numerous compounds either showed activities only in a concentration of 1 500 or were completely inactive

Differences in the sensitivity of the yeast and mycelial phase were

regularly observed Sulfathiazole sulfadiazine and sulfamethazine were inactive against mycelial growth but were active when the organism was maintained in the yeast phase at 37°C As seen from Table 1 *Histoplasma capsulatum* in the yeast phase was more sensitive against compounds which had only slight activity against the

TABLE 1

IN VITRO ACTIVITY OF VARIOUS SULFONAMIDES IN SYNTHETIC MEDIUM AT 25 C AND 37 C. INOCULUM 380 000 CELLS/ML.

Compound	25° C Mycelial Growth		37° C Yeast Phase	
	Complete Inhibition	Partial Inhibition	Complete Inhibition	Partial Inhibition
Sulfanilamide	1 1000	1 5000	1 1000	1 5000
Sulfathiazole	0	1 500	1 1000	1 10000
Sulfapyridine	1 500	1 1000	1 750	1 5000
Sulfadiazine	0	1 10000	1 500	1 10000
Sulfapyrazine	1 500	1 10000	1.500	1 10000
Sulfamethazine	0	0	1 1000	1 1000

Inactive Sulfamerazine sulfadimetin sulfisoxazole sulfathiourea sulfasuxidine

fungus growing in the mycelial phase Whether this is due to an enhanced sensitivity of the yeast form to sulfonamides or to an enhanced activity of the sulfonamides at higher incubation temperatures has not yet been established

The similarity of results obtained in both the Sabouraud s and the synthetic medium virtually devoid of PABA may be due to several facts namely (1) that amounts of PABA not detectable by chemical means interfere with sulfonamide activity (2) that the organism itself produces PABA (3) that PABA does not interfere with sulfonamides in the case of *H capsulatum* or (4) that factors other than PABA may interfere with the activity of sulfonamides

We have ascertained that PABA is formed by Histoplasma growing in the yeast phase with the use of the Bratton Marshall method. The amounts produced by the organism are very small the maximum concentration detected in cells being 0 02 mg per cent (Table 2) Compared with the relatively large quantities of PABA

TABLE 2
AMOUNTS OF PABA IN *HISTOPLASMA CAPSULATUM* AND OTHER MICRO-ORGANISMS FORMED IN SYNTHETIC MEDIUM

Micro-organism	*Amount of P A B A Formula (mg /7)*
Saccharomyces cerevisiae	0 26 6.3
Candida albicans	1 28
Staphylococcus aureus	0 01 0 220
Histoplasma capsulatum not lysed yeast phase	0 02
Histoplasma capsulatum broken by ultrasonic vibration yeast phase	0 02

produced by other micro-organisms particularly Candida Saccharomyces and certain Staphylococci this amount may seem negligible But they are apparently sufficient since PABA is capable of interfering with the sulfonamide action against Histoplasma in extremely minute concentrations as shown in Table 3 where the activities of three sulfonamides on Histoplasma in absence or presence of PABA are recorded In concentrations up to 1 5 million PABA completely nullifies the action of sulfanilamide and in a concentration of 1 50 million it nullifies that of sulfathiazole The anti sulfonamide action of PABA so strong in the case of sulfanilamide and sulfathiazole is much less pronounced in the case of sulfathiourea As shown in Table 4 these results corroborate an earlier finding by Mayer[1] that at least part of the antifungal and

TABLE 3

ACTION OF PABA UPON THE ANTIHISTOPLASMA ACTIVITY IN VITRO OF SULFANILAMIDE AND SULFATHIAZOLE

PABA	Dilutions of Sulfonamide $\frac{1}{500}$	$\frac{1}{1000}$	$\frac{1}{5000}$	$\frac{1}{10\,000}$	$\frac{1}{20\,000}$	PABA Control
Sulfanilamide	±	+	+	++	++	
plus PABA $\frac{1}{5000}$	++++	++++	++++	++++	++++	+
$\frac{1}{10\,000}$	++++	++++	++++	++++	+++	++
$\frac{1}{100\,000}$	++++	++++	++++	++++	++	+++
$\frac{1}{1\,m}$	+++	++++	+++	+++	+++	++++
$\frac{1}{10\,m}$	++++	++++	++++	++++	++	++++
$\frac{1}{100\,m}$	+++	+++	++	++	++	++++
$\frac{1}{1\,B}$	++	++	+	±	±	++++

antimycobacterial activity of sulfathiourea is due to the thiourea moiety which is not amenable to the action of PABA

IN VIVO EXPERIMENTS

Mice were infected with 40 million cells (yeast phase) by intra venous injection The average survival time was ten to twelve days after infection with a survival rate varying between 0 and 10 per cent on the thirtieth day after infection Treatment was started twenty four hours prior to infection and continued for fifteen days The sulfonamides were mixed with ground diet

TABLE 4

INFLUENCE OF PABA ON THE *IN VITRO* ACTIVITY OF SULFONAMIDES AGAINST *HISTOPLASMA CAPSULATUM*

Compound	$\frac{1}{500}$	$\frac{1}{1000}$	$\frac{1}{5000}$	$\frac{1}{10\,000}$	$\frac{1}{20\,000}$
PABA	0	+	++		
Sulfanilamide	*0*	*0*	++	++	+++
plus PABA $\frac{1}{1000}$	+++	++			
plus PABA $\frac{1}{5000}$	++++	++++	++++	++++	
Sodium sulfapyridine	*0*	+	+	++	++
plus PABA $\frac{1}{1000}$	+++	++			
plus PABA $\frac{1}{5000}$	++	+++	+++	+++	+++
Sulfathiourea	+	+	++	+++	+++
plus PABA $\frac{1}{1000}$	+	+	+	+	+
plus PABA $\frac{1}{5000}$	++	++	++	++	

0 indicates no growth

+ to ++++ indicates slight to massive growth.

RESULTS

Diet Experiments The results of the in vivo experiments are inscribed in Tables 5 and 6 All readily absorbed sulfonamides at the calculated daily oral intake of 25 to 50 mg per mouse yielded survival rates of 100 per cent with an average survival time of more than twenty days beyond that of the control group Sulfonamides demonstrating this activity were sulfanilamide sulfa

TABLE 5

IN VIVO ACTIVITY 1
MICE CONCENTRATION IN DIET FED 24 HOURS BEFORE TO 15 DAYS AFTER INFECTION INFECTION INTRAVENOUSLY 20 MILLION CELLS

Compound	In Vitro Activity 37° C	Percent Survival on 35th Day			
		90	100	50	Limit of Activity
Sulfanilamide	$\frac{1}{1000}$	1	*	0 25	0 15
Sulfathiazole	$\frac{1}{1000}$	1		0 25	<0 1
Sodium sulfathiazole	0	0 5			
Sulfapyridine				•	•
Sodium sulfapyridine	$\frac{1}{1000}$	0 5		0 1	<0 1
Sulfadiazine	$\frac{1}{10000}$ partial	0 25		0 1	<0 1
Sulfapyrazine	$\frac{1}{500}$	0 1		<0 1	
Controls	57 survival				

* Denotes concentration in diet

thiazole sodium sulfapyridine sulfamethazine sulfamerazine sulfadimethine sulfisoxazole and sulfathiourea Two other compounds sulfadiazine and sulfapyrazine were definitely more active producing 100 per cent survivals at doses of 5 to 10 mg per mouse Fifty per cent survival rates were obtained at daily doses below 5 mg with sodium sulfapyridine sulfadiazine sulfapyrazine sulfamethazine and sulfamerazine

Delayed Therapy These favorable results in experimental histoplasmosis of mice were unexpected since they were not in agree

TABLE 6
IN VIVO ACTIVITY 2

Compound	In Vitro Activity 37° C	Percent Survival on 30th Day			
		90	100	50	Limit of Activity
Sulfamethazine	$\frac{1}{1000}$	0 5	*	0 1	
Sulfamerazine	0	0 5		0 1	
Sodium sulfamerazine	$\frac{1}{1000}$	0 5			
Sulfadimetin	0	1 0			0 6
Sulfisoxazole	0	1		0 35	0 125
Sulfathiourea	0	0 5			0 1
Sulfathalidine	$\frac{1}{500}$	Very slight activity			
Sulfasuxidine	0	Inactive			

* Denotes concentration in diet

ment with the negative results obtained by Levy[2] with sulfanilamide and because of the poor results recorded in the literature in the treatment of generalized histoplasmosis in humans with sulfa drugs Since the sulfonamide therapy was instituted in our experiments twenty four hours prior to infection and then continued for fifteen days it was possible that our favorable results could be ascribed to a preventive rather than a curative effect of the sulfonamides employed In order to more closely simulate actual therapeutic use of a drug in a well established infection the initiation of the therapy was delayed for one to four days several days after the infection The results of this experiment using sulfadiazine and sulfathiourea are recorded in Table 7

It is evident from these data on delayed therapy that the sulfa

TABLE 7

DELAYED THERAPY
SULFADIAZINE AND SULFATHIOUREA
MEDICATED DIET PER CENT SURVIVALS ON 30TH DAY

Compound Concentration in Diet	Delay after Infection in Days				
	0	1	2	3	4
Sulfadiazine					
0 5%	100	100	100	100	70
0 1%	80	80	90	80	40
Sulfathiourea					
1%	100	100	70	40	20
0 5%	70	30	20	20	0
Controls	5				

drugs not only exert a prophylactic action but are definitely curative as well A daily oral intake of 25 mg of sulfadiazine still resulted in 100 per cent survival when delayed for three days following infection Five milligrams of sulfadiazine started on the third day after infection resulted in a survival rate of 80 per cent Sulfathiourea was less active in that 50 mg resulted in 100 per cent survival only when the delay did not exceed twenty four hours after infection This type of experiment is particularly useful for the detection of differences in the activity of two different compounds

VARIOUS ROUTES OF ADMINISTRATION

In the following experiment we have assessed the influence of the route of administration on the effectiveness of sulfonamide therapy Levy administered sulfonamide intraperitoneally and found it without effect whereas in our diet experiments it was highly effective We have therefore investigated to discover whether administration by the intraperitoneal route was less ef

fective even when higher therapeutic doses were employed In the experiment recorded in Table 8 groups of ten mice each were in

TABLE 8

ACTIVITY OF SULFANILAMIDE BY DIFFERENT ROUTES OF ADMINISTRATION PER CENT SURVIVAL AT VARIOUS DOSE LEVELS (PER MOUSE)

Route of Administration	*Dosages*			
	50 mg	25 mg.	7 5 mg	6 mg.
Diet	100	50	20	
Intraperitoneal		10* (twice daily)		0† (three times *daily*)

* Administered in divided doses

† Administered in three equal doses

fected and treated with medicated diets containing 1 0 5 and 0 1 per cent sulfanilamide corresponding to 5 25 and 50 mg daily oral intake respectively A second group of ten infected mice was treated intraperitoneally twice daily with 12 5 mg of sulfanila mide which corresponded to 25 mg in the above mentioned diet experiment A third group of ten infected mice received in three divided doses a total of 6 mg corresponding approximately in medicated food to 0 12 per cent the dose employed by Levy Whereas a daily dose of 50 mg of sulfanilamide was highly active when given in the diet resulting in 100 per cent survival on the thirtieth day a daily dose of 25 mg gave 50 per cent survival and a daily dose of 5 mg of sulfonamide still 20 per cent survival the same compound showed minimal activity when a total dose of 25 mg was injected intraperitoneally in two daily doses of 12 5 mg each and completely inactive at the dose employed by Levy of 18 mg per day

It was shown in previous experiments that the nitro sulfona mides have a higher activity against infections than has the corre

sponding amino sulfonamide [4,5] Nitro sulfathiazole showed considerably higher activity than the corresponding amino sulfathiazole (Table 9) Further experiments on the activity of nitro derivatives and other intermediates are under way

TABLE 9

AMINO SULFATHIAZOLE AND NITRO SULFATHIAZOLE COMPARISON OF IN VITRO AND IN VIVO ACTIVITY (PER CENT SURVIVAL)

Compound	In Vitro Inhibiting Concentration at 37° C	In Vivo Concentration in Diet 1% A*	1% B†	0 5% A	0 5% B	0 25% A	0 25% B	0 1% A	0 1% B
Amino sulfa thiazole	$\frac{1}{1000}$	100	21 9	85	17 1	50	12 7	50	10 9
Nitro sulfa thiazole	$\frac{1}{2500}$	100	21 4	100	21 4	90	20 5	90	20 7
Controls		5% survival							

* Per cent survival

† Increase in average survival time

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CHESTER W EMMONS

The Isolation of *Cryptococcus neoformans* from Pigeon Droppings

Although *Cryptococcus neoformans* is the cause of about 10 per cent of the 300 to 400 fatal mycoses reported annually in the United States [1] the nature of the primary disease and the sources of infection have remained obscure The familiar meningeal syndrome is known to have been preceded in some cases by a pulmonary lesion and it is probable that the primary lesion of cryptococcosis is usually in the lung [2] It may be that cryptococcosis is a frequent benign pulmonary disease and that the neurotropic predilection of the fungus is a rare manifestation Cryptococcosis is not contagious and we must look as we have learned to do in most systemic mycoses for an exogenous source of the infectious agent

C neoformans was actually first isolated as a saprophyte from fruit by Sanfelice in 1895 [3] Its isolation from a source unrelated to tissues exudates or surfaces of man or animals was not reported again until its isolation from soil in 1951 [4] Despite the paucity of evidence accumulated during the past sixty years recent observations have shown that the fungus is not rare in man s environment

Isolations of *C neoformans* from twenty specimens of soil and organic debris were reported in 1954 [5] Most of these specimens were collected from varied environments such as barnyards granaries and in and around chicken houses However in one collection *C neoformans* was isolated from eight of eleven specimens consisting of old pigeon nests and pigeon dung taken from the attic of a partially demolished house near Gaithersburg Maryland A systematic study was then undertaken to determine whether there is a frequent association between the saprophytic occurrence

of Cryptococcus and pigeon dung Collections were made in Lou doun County Virginia *

MATERIALS AND METHODS

Specimens were collected during the winter and early spring of 1955 and consisted of pigeon nests of the previous season and droppings taken from under the roosting places of pigeons The pigeon builds a flimsy nest of straw weed stems and twigs and raises sev eral broods during the season Unlike most birds it does not re move excreta from the nest so that as successive broods are raised the nest is transformed into a firm mortar like mass Therefore the nests as well as materials collected under roosting places were es sentially pigeon excreta in various stages of weathering and decay

From sixteen farm premises and three buildings in Leesburg one hundred and eleven specimens were collected The only basis for selection of premises was the observed presence of pigeons One location was a bell tower over the City Hall where an accumula tion of pigeon manure reached a depth of 3 inches Most of the collections were from stables and haymows on farms

Specimens were collected directly into 25 × 150 mm sterile tubes stored at 2 to 5 C until processed and suspended in physio logic saline solution A portion of the supernatant with added pen icillin and streptomycin was injected intraperitoneally into white mice The mice were killed three to four weeks later and cultures were made from the spleen and liver and incubated at 30 C This method with various modifications has been described in several papers[8 11]

The frequent isolation of *C neoformans* from dung suggested that the fungus may parasitize the tissues of the pigeon or at least be ingested with food and pass uneventfully through the digestive tract Twenty young pigeons including half grown birds and feath ered squabs nearly ready to fly were collected as they became avail able in the spring A host parasite relationship was sought by direct culture of the spleen liver and kidney of the birds Presence of the fungus in the digestive tract was sought by injecting separately

* I am indebted to Dr Charles G Souder Loudoun County Health Officer for his courtesy and cooperation in this study

the contents of the crop, gizzard upper intestine and lower intestine into the peritoneal cavity of mice as described above

It was possible in most cases to identify colonies of *C neoformans* by gross examination of primary cultures Usually subcultures were made on Sabouraud s agar containing penicillin and streptomycin to free the culture of contaminating bacteria In order to confirm the identifications isolates from twenty specimens were tested for their ability to produce acid in various sugars and isolates from the first forty seven positive specimens were tested for virulence in mice A forty eight hour culture was suspended in saline counted in a Levy chamber and adjusted by dilution so that 0 05 ml contained an estimated 10 000 cells This amount was then injected intracerebrally into four mice per isolate Mice were observed for illness death and development of intracranial lesions

RESULTS

C neoformans was isolated from sixty three of the one hundred and eleven specimens collected and from sixteen of the nineteen premises from which collections were made (Table I) From one farm none of five specimens yielded the fungus From another farm the pigeons had been driven away and the single specimen collected may have been sparrow and starling droppings From a building in Leesburg one specimen from an exposed roof gutter and a second consisting of a newly constructed nest were negative From the remaining sixteen premises *C neoformans* was isolated from 20 to 100 per cent of specimens collected From five farms every specimen collected yielded the fungus

Many of the mice injected intraperitoneally with specimens were killed after only three weeks Splenomegaly was observed in a few mice but for the most part no lesions which could be attributed to Cryptococcus were observed Cultures were usually contaminated with saprophytic bacteria or molds which survived the short incubation period in the mouse

The strains of *C neoformans* isolated from pigeon excreta exhibited the usual variation in encapsulation pigmentation and acid production noted in isolates from soil and human infections [2, 4] They produced in mice a typical dome shaped elevation

TABLE 1

ISOLATIONS OF *CRYPTOCOCCUS NEOFORMANS* FROM OLD PIGEON NESTS AND PIGEON DROPPINGS

Premises	*Specimens Collected*	*Cryptococcus Isolated*
Cla	5	5
Fra	5	5
Sto	5	5
Bis	5	5
Jen	1	1
Bel	10	9
Sol	6	5
Jac	5	4
Hor	4	3
Pax	13	9
LMS	5	3
Mch	10	3
Nor	4	1
LTH	10	2
Aha	10	2
Gal	5	1
Kir	1	0
LCB	2	0
Cor	5	0
19	111	63

of the skull and intracerebral lesions in which the encapsulated cells of Cryptococcus were easily demonstrated

Twenty birds have been examined to date without finding Cryptococcus either in the spleen liver or kidney of the bird by direct culture or in the digestive tract by mouse passage A fatal infection caused by *Trichomonas gallinae* regularly developed in mice receiving crop contents

DISCUSSION

The frequent isolation of C *neoformans* from old pigeon nests and droppings has revealed an important habitat of this pathogenic fungus unrecognized since its first isolation from a saprophytic source sixty years ago The association appears to represent

a fortuitous colonization of dung by the fungus A host parasite relationship with the pigeon is not to be expected because *C neo formans* does not grow well at the normal body temperatures of the bird It is reasonable to suppose that the pigeon may ingest contaminated food and that the fungus survives passage through the digestive tract but as yet this supposition has not been supported by evidence This point is still under investigation

The presence of nesting pigeons in barns is relevant to veterinary mycology Cryptococcosis occurs in cattle horses cats and other animals and two large epidemics of intractable bovine mastitis caused by *C neoformans* have been reported [12 14] Farmers generally consider pigeons a nuisance and dairy farmers in the area under study had made an effort to exclude them from dairy barns

An association between cryptococcosis in man and exposure to pigeon excreta has not been recognized The grave implications of such an exposure should be considered in future case studies and in appropriate environmental situations One of the premises from which *C neoformans* was isolated (nine of thirteen specimens) is an old estate (now used as a children s home) in which pigeons nest on ledges and cornices in the old residence and in stables and mows where children play Pigeons should be excluded from the grounds of an institution housing many children in order to reduce a hazard now recognized for the first time

Outbreaks of pneumonitis among persons exposed to accumulations of pigeon droppings while cleaning out belfries abandoned water towers or attics have been diagnosed in the past as ornithosis or histoplasmosis In some instances the diagnostic criteria have been incomplete or unsatisfactory The diagnosis of cryptococcosis must be considered in such epidemics

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ROBERT LUBARSKY and ORDA A PLUNKETT

Some Ecologic Studies of *Coccidioides immitis* in Soil

It is now accepted that coccidioidomycosis usually occurs as a pulmonary infection resulting from the inhalation of dust containing the arthrospores of *Coccidioides immitis* This plus the facts that the incidence of the disease is higher after sand and dust storms and that many researchers have been able to isolate the fungus from the soil led us to the following ecologic study of the organism in the soil

Since the disease is endemic in the southwestern United States the soils utilized in this first experiment were as follows sixteen from southern California and two from southeastern Arizona The soils varied from almost pure sand to rich loam to clay Attempts at isolation of *C immitis* from these soils were unsuccessful and it was concluded that they were free from the fungus Fifteen gram subsamples of each soil were placed in each of ten cotton stoppered test tubes to which an additional 2 ml of water was added The subsamples were divided into two sections one being sterilized and the other remaining in the unsterilized state This was done in order to see if any of the natural occurring organisms present in the soil had any inhibitory effect on the fungus All of the subsamples were then inoculated with 1 ml of a heavy suspension of fungal elements and incubated at room temperature

At the end of ten days the tubes containing the sterilized soil showed signs of fluffy white growth on the surface and in the air spaces between the soil particles At this time there was no visible evidence of growth in the nonsterilized soils At the end of fifteen days there was further evidence of growth in the tubes originally containing the sterilized soil and the beginning of visible growth in the nonsterilized soil At the end of thirty days all of the tubes

of inoculated soils revealed the presence of heavy fungal growth with the tubes originally containing the sterilized soils producing the most profuse growth The fungus growths were shown to be *C immitis* when inoculation of isolates produced the typical disease in mice and autopsy of these animals revealed the typical coccidioidal spherules

Some of the sandy soils were sifted in order to separate the sand from the bits of leaves and twigs The sand was placed in one set of test tubes and the twigs and leaves in another set When treated in the same manner as described above both the sand and the vegetative matter supported profuse fungal growth A single dry leaf when inoculated with an aqueous suspension of fungus elements produced profuse growth of *C immitis* for as long as moisture was present

Since the preceding experiment showed that the soils of southern California and Arizona could support the growth of *C immitis* it was decided to determine whether the soils from other areas of the United States could do likewise Soil samples were obtained from various areas of New York New Jersey Pennsylvania Oklahoma Colorado and Texas These soils were free from the fungus and when they were subjected to the same treatment as the soils described previously the results were almost identical In all cases the soils supported the growth of *C immitis* as long as moisture was present and the fungus could be isolated from these soils in a viable state for over eighteen months

Since all of the soils tested supported the growth of *C immitis* we decided to attempt to simulate some climatic conditions that is high and low temperatures and see what effect this might have on the survival of the fungus in the soil Soils obtained from the aforementioned areas were prepared in the same manner as described earlier in this paper and each soil sample was inoculated with a 1 ml of a heavy suspension of *C immitis* arthrospores and mycelial fragments These were then allowed to incubate at room temperature for a minimum of four days Half of these soil samples were exposed to a constant temperature of 42 C and the other half of the samples were exposed to freezing at minus 20 C and thawing at room temperature The period of alternate exposure to freezing and thawing was four days at a time for each type of exposure Both the freezing thawing and constant temperature

exposures were carried out over 129 days Viability checks were run every day on each soil sample and viable *C immitis* was isolated from each tube each time Two four day old agar cultures of the fungus were exposed to a constant temperature of 42°C and at the end of eight days of exposure were no longer viable

Realizing that the profuse growth of the fungus in the soil in the above experiments would result in a change of the soil pH the following check was made on the pH range that would support growth of the fungus on media Plates of Sabouraud s medium and Stewart and Meyer s medium were prepared with adjusted pH ranges of 3 0 to 9 5 and inoculated with *C immitis* The plates were incubated at room temperature and at the end of the forty eight hours there was evidence of growth in the plates in the pH range of 4 5 to 9 0 At the end of 120 hours growth was visible in pH range 3 5 to 9 0 with the most profuse growth at pH 6 5 to 7 5

It may be concluded from the aforementioned experiments that *C immitis* can grow and survive in soils ranging from rich loam to almost pure sand obtained from different parts of the United States Growth is not confined to the soil alone but can be and is supported by the dead vegetative matter normally found in soils The pH range at which the fungus will grow covers the pH ranges found in most soils Extremes of heat and cold have little effect on the viability of *C immitis* as long as the fungus is within the soil

ROGER O EGEBERG and ANN FRANK ELY

Coccidioides immitis in the Soil of the Southern San Joaquin Valley

In searching for the nidus of the mycelial phase of *Coccidioides immitis* one must consider among other possible sites mammals living and dead reptiles vegetation dung and the soil itself In order to throw light on the possible relationship between the animals and the soil and to learn more about the behavior of *C immitis* in nature we began several years ago to trap small mammals catch reptiles gather plant life and collect soil samples in the southwestern part of the San Joaquin Valley

This paper deals with the percentile recovery of *C immitis* from 500 soil samples collected between Buttonwillow and McKittrick

EXPERIMENTAL METHODS

Soil samples were collected in test tubes or jars from the walls of animal burrows and from the open areas between burrows At each random site — all being 5 or more feet away from the nearest burrow — four samples were taken one from the surface one 4 inches below the surface of the ground and one each at 8 and 12 inches below the surface One hundred and seventy seven burrow samples 93 random surface samples 78 samples 4 inches below the surface 77 samples 8 inches below the surface and 75 samples 12 inches below the surface were thus collected Three hundred and eighty of these samples were collected in mid January 1954 toward the end of the seven month dry season One hundred and twenty were collected in early April 1955 shortly after the end of the wet season and following several weeks of warm weather

In the laboratory the samples were handled as follows After one minute of shaking of each sample 1 gm was removed and sus

pended in 9 cc of sterile water in a test tube From this 1 10 dilution suspensions of 1 100 and 1 500 were made (In the case of the larger samples 10 gm was removed suspended and diluted proportionately) Plates of Sabouraud s fortified medium were inoculated in duplicate with 0 5 cc from each of the three dilutions thus making six plates from each soil sample The plates were allowed to incubate at room temperature for ten to twenty days and were then examined grossly All colonies that were suggestive of *C immitis* were examined microscopically Subcultures were made on straight Sabouraud s medium from those colonies whose microscopic morphology was typical of or similar to *C immitis* If after suitable incubation they looked typical grossly and microscopically material from the colony was inoculated into mice for observation of lesions and/or recovery of spherules the final proof that the fungus was *C immitis* No cultures were considered positive for *C immitis* unless the above criteria were met

RESULTS

From the first 500 soil samples tested in this analysis *C immitis* was recovered from 35 or 7 per cent This included random samples and burrow wall samples collected at the end of the dry and wet seasons

The distribution of *C immitis* was as follows 177 samples from animal burrows yielded 24 positive cultures or 13 6 per cent 323 random samples from the surface and varying depths yielded 11 positive cultures or 3 4 per cent (See Table 1)

TABLE 1

POSITIVE CULTURES

	Number of Samples	Positive Cultures No	%
From burrows	177	24	13 6
Random samples	323	11	3 4

Samples collected in the two major seasons were distributed as follows 380 were collected in mid January and 120 in mid April (Table 2) In the first group there were 16 positive samples or 4 2 per cent In the second group there were 19 positive samples or

TABLE 2

POSITIVE CULTURES AT END OF SEASON

	Number of Samples	Positive Cultures No	%
Collected at end of dry season	380	16	4 2
Collected at end of wet season	120	19	16 2

16 per cent In the first group 137 animal burrows yielded 10 positive cultures or 7 3 per cent Two hundred and forty three random samples from the surface and at 4 8 and 12 inch depths yielded 6 positive cultures or 2 5 per cent These positive cultures were found primarily below the surface As opposed to this the 120 samples taken at the end of the rainy season gave the following results Forty animal burrows yielded 14 positives or 33 3 per cent Eighty random samples taken from the surface and at 4 8 and 12 inch depths yielded 5 positives or 6 1 per cent In this instance the only positive cultures of the group were on the surface

DISCUSSION

The over all statistic of 7 per cent positive cultures from 500 soil samples corroborates the work of Plunkett and Lubarsky who found approximately 6 per cent of their soil cultures to be positive

In our series the striking statistical difference between the positive results found in the samples obtained from the walls of animal burrows and the samples taken at random seems to point to the animal burrows as offering something in the environment that was conducive to the growth and/or concentration of *C immitis* One could postulate many things in this connection Protection may have been afforded by burrows from the very high summer surface temperatures or from short torrential winter rains or the burrows might act primarily as collecting receptacles for the fungi picked up from the surface of the ground by the burrow tenants fur Conceivably the nitrogen content of the soil at the site of the burrow might be slightly higher and more conducive to growth Insects associated with the animals could play a part or organisms

carried by the animal might encourage the growth of *C immitis* Whatever the reason the statistical difference is significant and we are investigating this further

A comparison between the results obtained from soils collected at the end of the dry season and those collected at the end of the wet season deserves comment First the percentage of positive samples is appreciably higher at the end of the wet season Second the ratio of burrow samples that gave positive results to random samples that gave positive results in the two seasons is similar if not exactly proportionate and finally the location of the positive random samples in the two different seasons is interesting Of the 6 positive random soil samples procured at the end of the dry season only 1 came from the surface the others being from 4 8-, or 12 inch depths (Table 3) As opposed to this all of the positive

TABLE 3

POSITIVE CULTURES IN RANDOM SAMPLES

Source	*January*			*April*		
	No of Samples	*Positive Cultures*		*No of Samples*	*Positive Cultures*	
		No	*%*		*No*	*%*
Surface	73	1	1 3	20	5	25
4 inch depth	58	2	3 4	20	0	0
8 inch depth	57	1	1 7	20	0	0
12 inch depth	55	2	3 6	20	0	0

random samples collected at the end of the wet season came from the surface There were no positive random samples below the surface in this group

The paucity of positive results on the surface of the ground at the end of the dry season probably indicates the sterilizing effect of the sun s heat which for weeks on end keeps the top centimeter of soil above 125°F through the four or five hottest hours of each day The negative results in the subsurface specimens gathered at the end of the rainy season are more difficult to explain

We should like to suggest a possible explanation for the large percentage of positive surface samples among those collected at the end of the wet season It is known from the work of Plunkett and Lubarsky Smith and others that *C immitis* can be grown in ster

ilized soils with ease but only with considerable difficulty or careful nursing does it seem to grow in untreated or unsterilized soil Could it be that this scorching sterilizing summer heat is necessary to the life of *C immitis* in that it prepares a thin layer of relatively sterile soil which needs only moisture to make it susceptible to invasion by the fungi living below and adjacent to it? Could this heat be an ally of the organism by giving it for a brief period of time a growth medium not thoroughly contaminated with its antagonists and competitors?

D PAPPAGIANIS and G KOBAYASHI

In Vitro Inhibition of *Coccidioides immitis* by Antihistaminics*

Independent observations of Carson and Campbell[1] and Reiss and Caroline[2] indicated that several systemic fungi as well as dermatophytes could be inhibited in vitro by certain antihistamines Similar findings were reported by Fahlberg[3] Although the antihistamines appear to be therapeutically successful against superficial mycoses (see for example Sokoloff[4]) they have not been effective in experimental systemic fungus infections (Reiss and Caroline[5] Fahlberg[6])

In attempts to elucidate the mode of action of antihistamines Landis and Krop[7] noted a reversal of fungistatic effect of antihistamines by the addition of histamine but not histidine in cultures of *Trichophyton mentagropyhtes* On the other hand Reiss and Caroline[5] observed no reversal of antihistamine inhibition of *Blastomyces dermatitidis* by either histamine or histidine whereas addition of ashed neopeptone did neutralize the fungistatic effect

While Reiss and Caroline[2] and Chinn et al[8] have demonstrated inhibition of *Coccidioides immitis* by several antihistamines the present report deals with some aspects of the coccidioidostatic effect of two other antihistamines

MATERIALS AND METHODS

Two antihistamines Benadryl R † and Pyribenzamine R ‡ were initially tested but only the former was used thereafter since

* These investigations were conducted under the sponsorship of the Department of Bacteriology University of California and the Office of Naval Research

The opinions contained in this report are not to be construed as reflecting the views of the Navy Department or the Naval Service at large (Article 1-5? U S Navy Regulations 1919)

† The Benadryl ® was supplied by Parke Davis & Company

‡ Dr Frank A Tra ers of Ciba Pharmaceutical Products made Pyribenzamine ® available to us

it proved effective at a lower concentration Solutions were made up in saline adjusted to pH 7 3 and sterilized by Seitz filtration Inocula were saline suspensions of spores collected from cultures on 2 per cent glucose and 1 per cent yeast extract agar Approximately 5×10^4 cells per milliliter were seeded into the test mixtures Tests for viability were performed at the end of this time by dilution of the inhibited cultures to below inhibitory levels of the drug in 1 per cent glucose and 0 5 per cent yeast extract broth In those instances recorded as no growth viability tests proved that the cultures had not been sterilized by the antihistamine that is there was primarily fungistatic action

The horse and human sera used as test media were diluted with buffered saline adjusted to pH 7 3–7 4 and sterilized by Seitz filtration

RESULTS

The comparative levels of inhibition by Benadryl and Pyribenzamine of three strains of C immitis are shown in Table 1 There appeared to be no difference in susceptibility of these strains and as pointed out earlier Benadryl was the more effective compound

As a means of studying the possible mechanism of inhibition it

TABLE 1

COMPARATIVE INHIBITION OF THREE STRAINS OF *COCCIDIOIDES IMMITIS* BY TWO ANTIHISTAMINES

Molar Concentration	*Strain of C. immitis*		
	Silveira	*Cash*	*PW*
Benadryl			
0 0002	+	+	+
0 0004	−	−	−
0 0008	−	−	−
0 0016	−	−	−
Pyribenzamine			
0 0002	+	+	+
0 0004	+	+	+
0 0008	+	+	+
0 0016	−	−	−

+ denotes visible growth
− denotes inhibition of growth

TABLE 2

THE EFFECT OF CULTURE MEDIUM USED ON ANTIHISTAMINE INHIBITION OF *COCCIDIOIDES IMMITIS*

Molar Concentration of Benadryl	*CYE**	*Basal #?†*	*25 per cent Human Serum*
0 0001	+	+	+
0 0004	+	+	−
0 0016	+	+	−

* 1 per cent glucose and 0 5 per cent Difco yeast extract broth pH adjusted to 7 3
† One half strength synthetic medium of Roessler et al [9]

was believed most desirable to work in a chemically defined synthetic medium Table 2 indicates that a concentration of Benadryl which was inhibitory in the presence of serum was not so if tested in synthetic or glucose and yeast extract media The failure to observe inhibition in the yeast extract medium was at first thought to be referable to the presence of histidine or histamine like substances However these substances were not present in the synthetic medium Consequently our attention was drawn to the carbohydrate content of the yeast extract and synthetic media both of which contained 1 per cent glucose Although the range of concentrations of Benadryl tested was rather limited the essential effect of glucose addition as shown in Table 3 was to raise the minimum inhibitory concentration of antihistamine two to four fold

The effect of histamine and histidine on the inhibition is indicated in Table 4 Two other tests of reversal by histamine indi

TABLE 3

EFFECT OF ADDITION OF GLUCOSE ON INHIBITION OF *COCCIDIOIDES IMMITIS* BY BENADRYL IN SERUM MEDIUM

Molar Concentration of Benadryl	*50 per cent Human Serum*	
	Plus 1 per cent glucose	*No glucose add d*
0 002	−	−
0 001	−	−
0 0005	+*	−
0 00025	+	−
0 00012	+	+

Microscopically extensive hypha formation but many ungerminated spores

TABLE 4

REVERSAL OF ANTIHISTAMINE INHIBITION OF *COCCIDIOIDES IMMITIS* BY HISTAMINE AND HISTIDINE

Molar Concentration of Benadryl	*Saline*	*25 per cent Human Serum Plus*					
		Histamine molar concentr			*Histidine molar concentr*		
		0 0125	0 00125	0 00025	0 0125	0 00125	0 00025
0 0025	−	+	−	−	+	−	−
0 00025	+	+	+	+	+	+	+
0 00005	+	+	+	+	+	+	+
0 00000	+	+	+	+	+	+	+

cated that in the general range of equimolar concentration the histamine counteracted the effect of the antihistamine However the molar ratio of histamine to antihistamine was variable again indicating the need for more defined conditions than those provided by the complex serum medium

DISCUSSION

It is apparent that the molar concentration of Benadryl required for inhibition of *C immitis* in these experiments was lower than that reported by Reiss and Caroline [2] and that it conforms more to the inhibitory level of Phenergan (Chinn et al [8]) These discrepancies of inhibitory dosage might reflect differences in techniques employed

As to the nature of this inhibition it is of interest that differences have been observed in attempts to reverse the fungistasis We have observed a reversal of inhibition by histamine as did Landis and Krop [7] for *T mentagrophytes* But whereas histidine seemed ineffective for the latter workers we observed reversal by this amino acid Reiss and Caroline [8] ascribed to the mineral (ash) fraction of neopeptone the capacity to overcome the inhibition of *B dermatitidis* by trimeton maleate This presumably was due to replacement of metallic ion enzyme cofactors possibly bound by the antihistamine Added to this is our observation that addition of 1 per cent glucose to 50 per cent human serum raises the minimum inhibitory level of Benadryl This could imply interference with carbohydrate metabolism

It is interesting to note the studies of Jauregui and Goldner [10]

on the hypoglycemia induced by an antihistamine in humans and rabbits This effect appears referable to inhibition of glycogenolysis of the liver cells Should the relatively high rate of endogenous oxygen uptake by *C immitis* (Pappagianis [11]) implicate a glycogen like storage product the antihistamine inhibition of this fungus might likewise reflect impairment of intracellular glycogenolysis

It is evident from the foregoing that the mode of action of the antihistamines is far from clear Indeed there may be several biochemical sites of action of this group of compounds

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ANTONIO GONZÁLEZ OCHOA

Effectiveness of DDS in the Treatment of Chromoblastomycosis and of Mycetoma Caused by *Nocardia brasiliensis*

The drug 4 4-diamino-diphenyl sulfone (DDS) was synthesized by From and Wittmann in 1908 [1] although the first compound of this nature had been found by Mitscherlisch in 1834 [2] and described under the name of sulfobenzine the name sulfone having been contributed by Kolbe [3] The bacteriostatic action of the drug against Streptococcus in mice was demonstrated independently by Fourneau et al [4] and Buttle et al [5] Rist et al [6] and Feldman et al [7] found that DDS was active against *Mycobacterium tuberculosis* in vitro and against tuberculosis in the guinea pig At that time DDS was thought to be highly toxic and the mono- and di substituted derivatives were proposed as being less toxic These sulfonic derivatives were used therapeutically in pulmonary tuberculosis and in leprosy [8] but since the results were discouraging in tuberculosis and highly optimistic in leprosy their use remained restricted to leprosy

In a personal communication Latapi in 1946 [9] suggested the use of a di substituted sulfonic derivative (Diasone *) for the treatment of mycotic mycetoma and one year later we obtained good results with its use in the treatment of two cases of actinomycotic mycetoma caused by *Nocardia brasiliensis* [1] Arnold also [11] reported the cure of one case of cervicofacial actinomycosis after treatment with Diasone This author did not isolate the etiologic agent but it probably corresponded to *Actinomyces bovis* In a comparative in vitro study on the inhibitory action of sulfadiazine penicillin streptomycin and Promin † we found [12] that the

* Abbott Laboratories
† Pa ke Davis Laboratories

sulfonic derivative was the least active showing only a slightly inhibitory effect in high concentrations (1 2000) Bojalil and Shiels [13] studied the in vitro activity of Promin and Diasone against various Nocardiae and showed that only *N brasiliensis* was sensitive

In spite of the supposed toxicity of DDS Floche and Destombes used the drug in leprosy and did not find a higher incidence of side effects than produced by its sulfonic derivatives Two years later in a study of the use of DDS and other therapeutic agents in patients with leprosy we came to the conclusion [14] that the active parent DDS was superior to its derivatives and since smaller daily doses were required it could be given orally Its toxicity was no greater than that of the sulfonic derivatives and it was economical in price

With this in mind we started in 1947 to use DDS * instead of its sulfonic derivatives for actinomycotic mycetoma caused by *N brasiliensis* a species that is the etiologic agent in 98 per cent of the mycetomas caused by aerobic Actinomycetes We performed in vitro studies against the aerobic Actinomycetes which cause deep mycoses and found that *N brasiliensis* alone was sensitive its growth being partially inhibited at 1 50 000 and completely inhibited at 1 10 000 [10] In the same paper we presented the results of treatment with DDS both in experimental infections of mice with *N brasiliensis* and in 8 cases of actinomycotic mycetoma in man caused by the same Nocardia These clinical cases have been observed for more than three years In the experimental infections the results were not conclusive but in the series of 8 patients we obtained very encouraging results Six patients were cured In a second paper [15] we reported our results with DDS in the treatment of patients having hitherto fatal thoracic mycetomas with pulmonary involvement and were able to reconfirm the effectiveness of this therapeutic agent

Since that time we have administered DDS to more than 100 patients but since the majority were agricultural workers and non cooperative patients it was not possible to observe them for a prolonged period of time However we consider those patients who

* The DDS used in this research was supplied in part by the Squibb Institute for Medical Research under the trade name of "Fenamum" The Institute also donated a grant which covered part of the expenses

have not returned as cured because in those instances in which relapses did occur the patient has returned for additional therapy Therefore we believe that the majority of patients are still free of symptoms

In Table 1 we present the results on 21 patients whom we observed for four years Of these patients 15 were cured and the other 6 patients experienced such marked improvement that they were considered to be clinically cured These 6 patients whether due to premature suspension of treatment or to the development of resistance suffered relapses which did not respond to subsequent treatment This series of patients confirms the conclusions of our first report[10] However we now consider it advisable to prolong duration of treatment

Those aspects of treatment which have a bearing on the results obtained may be listed as (1) dosage (2) duration of treatment (3) curability in relation to the type of tissue involved (4) relapses and (5) development of resistance

(1) *Dosage* The total dose of 200 mg a day one 100 mg tablet after breakfast and the other after dinner is recommended This average dose has been proposed after trying doses varying from 100 to 400 mg daily This is purely empirical and there is no correlation of dosage with blood levels With 200 mg daily the blood concentration varies from 0 26 mg /cc to 0 88 mg /100 cc which is ten times less than 5 mg /100 cc the amount required to partially inhibit *N brasiliensis* in vitro

(2) *Duration of Treatment* Both clinical cure and absolute or radical cure must be considered Clinical cure occurs early After one year of treatment the ulcers heal the fistulas close leaving hyperchromic depressed scars the nodules disappear and the tumors are reduced in size During this time improvement is also reflected in the laboratory findings The high sedimentation rate and the leukocytosis with neutrophilia which are present in the active stage of the disease also return to normal There is no way of determining whether the mycetoma has been actually cured or whether a latent focus exists However if treatment is suspended early relapses may occur and to provide a margin of safety DDS should be administered for two to three years after clinical recovery is obtained If treatment is suspended prematurely the patient may not show improvement again when the drug is reinstituted at a subse-

TABLE 1

ACTINOMYCOTIC MYCETOMA (*NOCARDIA BRASILIENSIS*) TREATED WITH DDS

Patient		Type of Disease	Duration	Results	
				Clinical Cure Obtained after	Definite Cure or Relapse after
1	FG	Dorsal localized in soft tissues	4 yr	14 mo	3 yr cured
2	RS	Axillary localized in soft tissues	11 mo	10 mo	2 yr cured
3	RP	Thoracic with lung involvement	13 mo	12 mo	3 yr cured
4	JR	Knee with joint involvement	2 yr	14 mo	3 yr cured
5	JS	Shoulder with bone involvement	18 mo	12 mo	2 yr cured
6	SS	Leg with bone involvement	3 yr	16 mo	3 yr cured
7	FD	Thoracic without lung involvement	1 yr	12 mo	2 yr cured
8	RL	Lower limb with bone involvement	6 yr	16 mo	3 yr cured
9	LM	Foot with bone involvement	4 yr	18 mo	3 yr cured
10	GJ	Thoracic with pulmonary involvement	3 yr	7 mo	After 13 mo became resistant and died
11	JZ	Foot and leg with bone involvement	4 yr	15 mo	3 yr cured
12	SM	Leg and thigh with bone involvement	6 yr	12 mo	After 16 mo stopped treatment became resistant and died
13	PV	Arm and forearm without bone involvement	2 yr	10 mo	2 yr cured
14	FP	Leg and thigh with bone involvement	2 yr	12 mo	After 18 mo stopped treatment and became resistant
15	VM	Foot with bone involvement	4 yr	11 mo	3 yr cured
16	RP	Thoracic with pulmonary involvement	2 yr	2 yr	4 yr cured
17	JR	Thoracic with pulmonary involvement	3 yr	16 mo	After 2 yr became resistant and died
18	JM	Foot and leg without bone involvement	1 yr	8 mo	2 yr cured
19	SL	Thoracic with lung involvement	3 yr	2 yr	Stopped treatment became resistant and died
20	JG	Foot with bone involvement	4 yr	14 mo	3 yr cured
21	AS	Arm and forearm without bone involvement	2 yr	8 mo	Stopped treatment after 1 yr became resistant

quent date This was the case in patients 12 14 19 and 21 as listed in Table 1 Amputation is then indicated if the mycetoma is localized to a limb and if surgical measures are not possible death occurs

(3) *Curability in Relation to Type of Tissue Involved* As a general rule the prognosis of any disease is closely related to the duration and extent of the lesions But in mycetoma the type of tissue involved is as important as and perhaps even more important than the duration and extent of the lesions Those located in soft tissues which are easily irrigated are more readily cured and two years of treatment are sufficient to obtain a radical cure (Cases 2 7 13 and 18) When the infection involves bones — and this happens in the majority of cases which are localized to the lower extremities — it is necessary to prolong the treatment for more than two years In the case of thoracic mycetoma the infection sooner or later extends into the lung producing pulmonary condensations with small cavities and eventually involves the vertebrae and spinal cord producing distressing neurologic complications The thoracopulmonary mycetoma has a poor prognosis but in some cases it is possible to obtain a complete cure (Cases 3 and 16) When the central nervous system is involved the prognosis is hopeless and in spite of high dosages of DDS little effect is observed and the disease terminates fatally At autopsy the cutaneous and muscular lesions are usually healed but the pulmonary and vertebral lesions show little evidence of healing Cases 10 and 17 were representative of this condition

(4) *Relapses* Relapses are due principally to the premature suspension of treatment We have mentioned that there are no criteria by which one can consider the patient to be completely cured The discussion of the curability index in the previous paragraph can be utilized as a pattern to provide information for determining the duration of treatment

(5) *Development of Resistance* In addition to the resistance that appears as a result of early suspension of treatment we have observed that when Nocardia infects the lung (Cases 10 and 17) it continues to spread until it produces an impairment of vital functions and death ensues even though treatment has been continued without interruption When both of these conditions occur simultaneously — that is early suspension of treatment and

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Patient	Type of Disease	Duration	Results	
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6 SS	Leg with bone involvement	3 yr	16 mo	3 yr cured
7 FD	Thoracic without lung involvement	1 yr	12 mo	2 yr cured
8 RL	Lower limb with bone involvement	6 yr	16 mo	3 yr cured
9 LM	Foot with bone involvement	4 yr	18 mo	3 yr cured
10 GJ	Thoracic with pulmonary involvement	3 yr	7 mo	After 13 mo became resistant and died
11 JZ	Foot and leg with bone involvement	4 yr	15 mo	3 yr cured
12 SM	Leg and thigh with bone involvement	6 yr	12 mo	After 16 mo stopped treatment became resistant and died
13 PV	Arm and forearm without bone involvement	2 yr	10 mo	2 yr cured
14 FP	Leg and thigh with bone involvement	2 yr	12 mo	After 18 mo stopped treatment and became resistant
15 VM	Foot with bone involvement	4 yr	11 mo	3 yr cured
16 RP	Thoracic with pulmonary involvement	2 yr	2 yr	4 yr cured
17 JR	Thoracic with pulmonary involvement	3 yr	16 mo	After 2 yr became resistant and died
18 JM	Foot and leg without bone involvement	1 yr	8 mo	2 yr cured
19 SL	Thoracic with lung involvement	3 yr	2 yr	Stopped treatment became resistant and died
20 JG	Foot with bone involvement	4 yr	14 mo	3 yr cured
21 AS	Arm and forearm without bone involvement	2 yr	8 mo	Stopped treatment after 1 yr became resistant

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pulmonary invasion — subsequent resistance becomes even more striking We have observed different degrees of sensitivity in the same organism isolated when the patient is improving under DDS treatment and in the isolates obtained when the lesions did not respond to treatment

SIDE EFFECTS

In spite of the long duration of DDS administration the serious toxic phenomena described in the literature were not observed The commonest side effect was a moderate normocytic iron deficiency anemia which was not difficult to control or to prevent by the administration of iron Three patients developed dermatologic eruptions as well as conjunctivitis dizziness and nausea In 2 of the patients the eruption was polymorphous and in the other it was papular These patients were all receiving a DDS preparation from a single lot made in Europe No dermatologic reactions were encountered with the use of Fenamum and these patients were able to continue treatment with this medicament

MODE OF ACTION

We do not know how DDS acts It is difficult to explain the results obtained by its fungistatic activity As we have mentioned[10] there is no relationship between the blood levels attained and the amount required in vitro to inhibit *N brasiliensis* In addition the experiment in which guinea pigs were infected with *N brasiliensis* and treated with doses of 300 200 and 100 mg /kg during an average of two months was not conclusive[10] Furthermore the effectiveness of DDS in the treatment of chromoblastomycosis also mitigates against the hypothesis that DDS acts primarily as a fungistatic agent

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FIGURE 1

Clinical condition at admission in January 1952



FIGURE 2

X ray photograph showing bone lesions

H SEELIGER and M REIFFERSCHEID

Successful Treatment of Mycetoma Caused by *Monosporium apiospermum*

Mycetoma caused by *Monosporium apiospermum* or *Allescheria boydii* and related fungi have been reported from various parts of the world The disease is chronic lasting sometimes for several decades Although it usually does not endanger life it disables the patient progressively and may eventually end fatally To our knowledge the only successful treatment so far reported is drastic surgery in which all of the infected area is removed

CASE REPORT

In January 1952 a 57 year old housewife who had never been outside of Germany was admitted to the Surgical Clinic Univer sity of Bonn with the diagnosis of a therapy resistant actinomy cosis

In 1926 the patient had suffered an intragluteal injury when she fell into a privet bush on her buttocks Although the superficial lesions healed rapidly she continued to have pain at the site of in jury radiating over the buttocks and the right upper thigh It was assumed to be rheumatic and was treated with salicylates

Nineteen years later in 1945 the patient again fell on her but tocks She suffered no external lesions however the right thigh and intragluteal area became swollen and inflamed There were intermittent attacks of spiking fever In the inflamed and indu rated region fistulas developed with pus formation From one of them a wooden splinter 1 cm in diameter apparently introduced at the first injury was recovered However the patient s condition did not improve From 1945 the area of inflammation and fistula formation increased steadily A diagnosis of actinomycosis was made in 1951 when white granules were found in the pus So far

FIGURE 1

Clinical condition at admission in January 1952



FIGURE 2

X ray photograph showing bone lesions

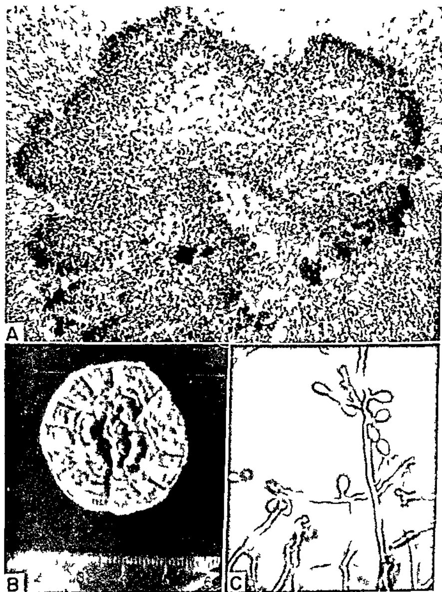


FIGURE 3

A White granule found in pus stained with methylene blue

B Macroscopic appearance of the isolated strain of *M. apiospermum* after 2 weeks at 37 C on Sabouraud's agar (V61/52)

C Slide culture of the isolated strain of *M. apiospermum* (V61/52)

as we know this diagnosis was based only upon the demonstration of granules not upon cultural findings

The patient was then treated without effect by oral administration of 150 gm of sulfonamides by injection of 52 million units of penicillin and finally by application of 20 gm of aureomycin She was transferred to our service in January 1952

On admission the patient showed the typical picture of a well developed mycetoma involving the right upper thigh buttocks part of the intragluteal area and the pubic bone

In the pus of the multiple fistulas and in biopsy material taken under aseptic precautions numerous white granules could be seen from which pure cultures of *M apiospermum* were grown repeatedly at 22 and 37 C on Sabouraud s agar (Figs 3a b c)

The patient s serum agglutinated at 1 40 a conidial suspension prepared from three isolates (V 64 1417 1994/52) and from comparative strains of *M apiospermum* and *A boydii* * The serum also contained specific precipitins and complement fixing antibodies (titer 1 32) for antigens of *M apiospermum A boydii* Intracutaneous tests with crude culture filtrates and partially purified polysaccharide extracts of *M apiospermum A boydii* gave strong positive reactions after twenty four to forty eight hours

Since the serologic results were identical with those obtained with a hyperimmune rabbit serum they were considered as expression of the immunologic response of the patient Negative results were obtained regularly with normal control sera from man and rabbit Comparative tests with a great number of antigens from other pathogenic and nonpathogenic fungi were consistently negative Details of this study are reported elsewhere (see page 332)

On admission the hemoglobin was 70 per cent with 3 600 000 erythrocytes and 21 000 leukocytes mm * per cubic millimeter The blood sedimentation rate was 125/131 and the patient had septic temperatures

On account of the above findings the diagnosis of a mycetoma due to *M apiospermum* (monosporiosis) was made

THERAPY

Massive sulfonamide and antibiotic therapy blood transfusions and x ray treatment had no apparent effect Due to iodine sensi

* Kindly furnished by Dr Conant and Dr A de Leão

tivity the administration of potassium iodide had to be discontinued The recovery of the wooden splinter and repeated attempts at surgical cleaning did not result in any noteworthy improvement but radical surgery could not be performed because of the involvement of the pubic bone

Therefore the fungistatic action of several chemical compounds used in the therapy of dermatomycoses was tested in vitro against six strains of *M apiospermum A boydii* Throughout these experiments Sabouraud s agar was used The compounds were mixed with the liquefied medium giving dilutions of 1 1000 to 1 500 000 Plates were poured and their surface was seeded with a heavy inoculum of conidia prepared from ten day old cultures of *M apiospermum A boydii* grown at 37°C The results were read after five days at 37°C

Of the compounds tested D25 (2 2 Dioxy 5 5 Dichlordiphenyl sulfide) * — which had been recommended by Richter and coworkers for dermatologic use — showed a complete inhibition of the growth of *M apiospermum A boydii* up to a dilution of 1 200 000 Full growth occurred in a similar test with a 1 100 dilution of the solvent of D25 The fungistatic effect was reduced to the 1 25 000 dilution of D25 by addition of 25 per cent ascitic fluid to Sabouraud s medium

No fungicidal effect could be observed when suspensions of *M apiospermum* conidia were mixed with dilutions of D25 ranging from 1 100 to 1 10 000 incubated for seven days at 37°C and then plated

These findings and previous investigations by Richter showing that the drug was tolerated by man indicated that the administration of this compound might be helpful for our patient

Two preparations of the drug were made available in 5 gm tablets in diacetylated form and in oil 0 5 pro injection

Daily oral administration of 6 gm divided in four doses was started in April 1952 After three and four weeks therapy no effective blood level could be found by the following method

Five milliliters of venous blood were drawn citrated and mixed with 5 ml of double strength liquefied Sabouraud s agar cooled to 50°C After hardening the surface of this medium was inocu

* Obtained from Boehringer und Soehne Mannheim Germany

lated with conidia of the patient s strain of *M apiospermum* Vigorous growth occurred within five days incubation at 37°C

At this time the D25 in oil became available Injection of 2 5–5 0 ml (corresponding to 0 5 and 1 0 gm) was made directly into the infected area at two- to three-day intervals Care was taken that within a period of three months all parts of the mycetoma were infiltrated

At first the oral administration was tolerated well but after six weeks toxic symptoms such as loss of appetite nausea abdominal cramps and palpitation necessitated reduction of the daily dosage from 6 gm to 3 gm Within eleven months 560 gm of the dia cetylated D25 was taken orally without any untoward effect on the blood picture or the nervous system

The intramuscular infiltration therapy was tolerated without any toxic symptoms or untoward tissue reactions A total of 110 ml of the oil emulsion was injected over a period of six months

RESULTS

Although the patient s clinical condition improved somewhat after the first four weeks of the oral D25 treatment no fungistatic blood level was demonstrable Whether this was due to a lack of absorption of the compound from the intestinal tract or to inac tivation by the blood is not known

Shortly after the intramuscular therapy was begun rapid and dis tinct improvement was noted The fistulas closed spontaneously the area of induration became softer and painless and the inflam mation disappeared The leukocyte count slowly dropped to 12 000 and later to 8000 and the erythrocyte count rose to 4 000 000 The blood sedimentation rate decreased to 60 in the first hour One month after the intramuscular therapy was started the patient was able to walk In July six months after admission she was discharged to her home but failed to continue therapy as advised

In the following September a new subcutaneous abscess de veloped and formation of a fistula was noted After readmission to the clinic another three months course of combined oral and in tramuscular D25 treatment was given In December the patient re turned to her home in satisfactory condition with no fistulas or any

signs of an active mycotic infection The serum reactions were at that time still positive with a slight drop in titers

FOLLOW UP

At the time of this writing the patient was being observed by her physician and no sign of an active mycotic infection has been noted since the end of 1952 In fact this possibility was flatly denied by another well known clinic where the patient was sent on account of increasing pain predominantly involving the sciatic nerve This pain was obviously due to extensive scar tissue formation in the treated area The scar tissue surrounded the sciatic nerve and its radicles

CONCLUSIONS

A case of mycetoma caused by *M apiospermum* the first known case in Germany of twenty six years duration and six years active progression was successfully treated by oral and local administration of D25 The local treatment apparently had a decisive effect and had to be continued for a period of six months In the follow up period of two years no sign of recurrence of the active mycotic infection has been noted

New data on the immunology of monosporiosis were obtained

Details of the above findings will be reported in the following publications

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Index of Contributors

Index of Subjects